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(54) Title: IDENTIFICATION OF GENETIC MARKERS OF BIOLOGICAL AGE AND METABOLISM

(57) Abstract: A method of measuring the biological age of a multicellular organism is disclosed. In one embodiment this method comprises the steps of obtaining a sample of nucleic acid isolated from the organism's organ, tissue or cell and determining the expression pattern of a panel of sequences within the nucleic acid that have been predetermined by either increase or decrease in response to biological aging of the organ, tissue or cell. A method of obtaining biomarkers of aging is also disclosed. This method comprises the step of comparing a gene expression profile of a young multicellular organism subject's organ, tissue or cells; a gene expression profile from a chronologically aged subject's organ, tissue or cell; and a gene expression profile from a chronologically aged but biologically younger subject's organ, tissue or cell and identifying gene expression alterations that are observed when comparing the young subjects and the chronologically aged subjects and are not observed or reduced in magnitude when comparing the young subject and the chronologically aged but biologically younger subjects.

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IDENTIFICATION OF GENETIC MARKERS OF BIOLOGICAL AGE AND METABOLISM

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to provisional application 60/148,540, filed August 12, 1999, U.S. provisional application 60/178,232, filed January 26, 2000 and 60/211,923 filed June 16, 2000. These provisional applications are incorporated by reference as if fully set forth herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with United States government support awarded by the following agencies: NIH Grant No: AG11915. The United States has certain rights in this invention.

BACKGROUND OF THE INVENTION

A common feature of most multicellular organisms is the progressive and irreversible physiological decline that characterizes senescence. Although genetic and environmental factors can influence the aging process, the molecular basis of senescence remains unknown. Postulated mechanisms include cumulative damage to DNA leading to genomic instability, epigenetic alterations that lead to altered gene expression patterns, telomere shortening in replicative cells, oxidative damage to critical macromolecules and nonenzymatic glycation of long-lived proteins (S.M. Jazwinski, Science 273:54, 1996; G.M. Martin, et al., Nature Gen. 13:25, 1996; F.B. Johnson, et al., Cell 96:291, 1996; K.B. Beckman and B.N. Ames, Physiol. Revs. 78:547, 1998). Factors which contribute to the difficulty of elucidating mechanisms and testing interventions include the complexity of organismal senescence and the lack of molecular markers of biological age (biomarkers). Aging is complex in that underlying mechanisms in tissues with

limited regenerative capacities (e.g., skeletal and cardiac muscle, brain), which are composed mainly of postmitotic (non-dividing) cells, may differ markedly from those operative in proliferative tissues. Accordingly, approaches which provide a global assessment of senescence in specific tissues would greatly increase understanding of the aging process and the possibility of pharmaceutical, genetic or nutritional intervention.

Genetic manipulation of the aging process in multicellular organisms has been achieved in *Drosophila*, through the over-expression of catalase and Cu/Zn superoxide dismutase (W.C. Orr and R.S. Sohal, Science 263:1128, 1994; T.L. Parkes, et al., Nat. Genet. 19:171, 1998), in the nematode *C. elegans*, through alterations in the insulin receptor signaling pathway (S. Ogg, et al., Nature 389:994, 1997; S. Paradis and G. Ruvkun, Genes Dev. 12:2488-2498, 1998; H.A. Tissenbaum and G. Ruvkun, Genetics 148:703, 1998), and through the selection of stress-resistant mutants in either organism (T.E. Johnson, Science 249:908, 1990; S. Murakami and T.E. Johnson, Genetics 143:1207, 1996; Y.J. Lin, et al., Science 282:943, 1998). In mammals, there has been limited success in the identification of genes that control aging rates. Mutations in the Werner Syndrome locus (WRN) accelerate the onset of a subset of aging-related pathology in humans, but the role of the WRN gene product in the modulation of normal aging is unknown (C.E. Yu, et al., Science 272:258, 1996; D.B. Lombard and L. Guanrente, Trends Genet. 12:283, 1996).

In contrast to the current lack of genetic interventions to retard the aging process in mammals, caloric restriction (CR) appears to slow the intrinsic rate of aging (R. Weindruch and R.L. Walford, The Retardation of Aging and Disease by Dietary Restriction (CC. Thomas, Springfield, IL, 1988; L. Fishbein, Ed., Biological Effects of Dietary Restriction (Springer-Verlag, New York, 1991; B.P. Yu, Ed., Modulation of Aging Processes by Dietary

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Restriction (CRC Press, Boca Raton, FL 1994). Most studies have involved laboratory rodents which, when subjected to a long-term, 25-50% reduction in calorie intake without essential nutrient deficiency, display delayed onset of age-associated pathological and physiological changes and extension of maximum lifespan.

BRIEF SUMMARY OF THE INVENTION

The present invention will allow the evaluation of aging interventions on a molecular and tissue-specific basis through the identification of aging biomarkers. In particular, the use of gene expression profiles allows the measurement of aging rates of target organs, tissues and cells, and to what extent aging is delayed by specific interventions, as determined by quantitative analysis of mRNA abundance. Because aging-related gene expression profiles can be classified in subgroups according to function, the invention also allows for the determination of how function-specific aspects of aging are affected. This particular feature will allow for determination of combination therapies that prevent or reverse most aging related changes in particular organs, tissues, and cells.

In one embodiment, the present invention is a method of measuring the biological age of a multicellular organism comprising the steps of (a) obtaining a sample of nucleic acid isolated from the organism's organ, tissue or cell, wherein the nucleic acid is RNA or a cDNA copy of RNA and (b) determining the expression pattern of a panel of sequences within the nucleic acid that have been predetermined to either increase or decrease in response to biological aging of the organ, tissue or cell. Preferably, the expression patterns of at least ten sequences are determined in step (b) and the organism is a mammal, most preferably a rodent.

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In one preferred embodiment of the method described above, the nucleic acid is isolated from a mammalian tissue selected from the group consisting of brain tissue, heart tissue, muscle tissue, skin, liver tissue, blood, skeletal muscle, lymphocytes and mucosa.

In another embodiment the present invention is a method of obtaining biomarkers of aging comprising the steps of: (a) comparing a gene expression profile of a young multicellular organism subject's organ, tissue or cells; a gene expression profile from a chronologically aged (and therefore biologically aged) subject's organ, tissue or cell; and a gene expression profile from a chronologically aged but biologically younger subject's organ, tissue or cell, and (b) identifying gene expression alterations that are observed when comparing the young subjects and the chronologically aged subjects and are not observed or reduced in magnitude when comparing the young subjects and chronologically aged and biologically younger subjects. Preferably, one uses high density oligonucleotide arrays comprising at least 5-10% of the subject's gene expression product to compare the subject's gene expression profile, and caloric restriction to obtain a chronologically aged but biologically younger subject.

In a preferred embodiment of the method described above, the gene expression profile indicates a two-fold or greater increase or decrease in the expression of certain genes in biologically aged subjects. In a more preferred embodiment of the present invention, the gene expression profile indicates a three-fold or greater or, most preferably three-fold or greater, increase or decrease in the expression of certain genes in aged subjects.

In another embodiment, the present invention is a method of measuring biological age of muscle tissue comprising the step of quantifying the mRNA abundance of a panel of biomarkers selected from the group consisting of markers described in the Tables 1, 2, 15 and 16. A method of

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measuring biological age of brain tissue comprising the step of quantifying the mRNA abundance of a panel of biomarkers selected from the group consisting of markers described in Tables 5, 6, 9, 10, 11, 12, 13 and 14.

In another embodiment, the present invention is a method for screening a compound for the ability to inhibit or retard the aging process in a multicellular organism tissue, organ or cell, preferably mammalian tissue, organ or cell, comprising the steps of: (a) dividing test organisms into first and second samples; (b) administering a test compound to the organisms of the first sample; (c) analyzing tissues, organisms and cells of the first and second samples for the level of expression of a panel of sequences that have been predetermined to either increase or decrease in response to biological aging of the tissue, (d) comparing the analysis of the first and second samples and identifying test compounds that modify the expression of the sequences of step (c) in the first sample such that the expression pattern is indicative of tissue that has an inhibited or retarded biological age.

It is an object of the present invention to evaluate or screen compounds for the ability to inhibit or retard the aging process.

It is also an object of the present invention to measure the biological age of a multicellular organism, such as a mammal in a tissue or cell-specific basis.

It is also an object of the present invention to obtain biomarkers of aging.

Other objects, features and advantage of the present invention will become apparent to one of skill in the art after review of the specification and claims.

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DETAILED DESCRIPTION OF THE INVENTION

One of the major impediments to the development of pharmaceutical, genetic or nutritional interventions aimed at retarding the aging process is the lack of a molecular method for measuring the aging process in humans or experimental animals. A suitable biomarker of the aging process should reflect biological age (physiological condition) as opposed to chronological age. Additionally, the biomarker should be amenable to quantitation, and reflect aging-related alterations at the molecular level in the tissue under study. Importantly, any such biomarker must be validated with the use of a model of retarded aging.

Caloric restriction, when started either early in life or in middle-age, represents the only established paradigm of aging retardation in mammals. (R. Weindruch and R.L. Walford, "The Retardation of Aging and Disease by Dietary Restriction" (C.C. Thomas, Springfield, IL, 1988)) The effects of caloric restriction on age-related parameters are broad: caloric restriction increases mean and maximum lifespan, reduces and delays both spontaneous and induced carcinogenesis, almost completely suppresses autoimmunity associated with aging, and reduces the incidence of several age-induced diseases. (R. Weindruch and R.L. Walford, supra, 1988) Therefore, we expect that the rate of change of most proposed aging biomarkers should be retarded by caloric restriction.

By "biological age" we mean the physiological state of an animal or tissue relative to the physiological changes that occur throughout the animal's lifespan. By "chronological age" we mean the age of an animal as measured by a time scale such as month or years.

Because gene expression patterns are responsive to both intracellular and extracellular events, we reasoned that simultaneous monitoring of thousands of genes on a tissue-specific or organ-specific basis would reveal

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a set of genes that are altered in expression levels as a consequence of biological aging. Although alterations in gene expression with aging had been previously investigated for some genes, a global analysis of gene expression patterns during aging, and the validation of such patterns as a tool to measure biological age through the use of a model of retarded aging had not been previously performed. Such global analysis is required to identify genes that are expressed differentially as a consequence of aging on different cell types that compose the tissue under study and will allow a quantitative assessment of aging rates.

There exists a large and growing segment of the population in developed countries that is suffering from age-associated disorders, such as sarcopenia (loss of muscle mass), neurodegenerative conditions, and cardiac disease. Therefore, the market for compounds that prevent aging-associated disorders and improve quality of life for the elderly is likely to drive research and development of novel drugs by the pharmaceutical industry. As an example, many drugs, nutraceuticals and vitamins are thought to influence aging favorably, but their use remains limited due to the lack of FDA approval. The inability to assess biological aging in tissues at the molecular level precludes proper animal and human testing of such compounds.

In one embodiment, the invention is a method for measuring the biological aging process of a multicellular organism, such as a mammal, at the organ, tissue or cellular level through the characterization of the organism's gene expression patterns. This method preferably comprises obtaining a cDNA copy of the organism's RNA and determining the expression pattern of a panel of particular sequences (preferably at least 5 sequences, most preferably at least 10 sequences and more preferably at least 20, 30, 40, or 50 sequences) within the cDNA that have been predetermined to either increase or decrease in response to biological aging

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of the organ, tissue or cell. (We refer to nucleotide sequences with alterartions in expression patterns characteristic of biological age as "biomarkers.") One may characterize the biological age of the organism by determining how many and at what level the biomarkers are altered.

Tables 1-4 and 15-16 describe a specific gene expression profiles determined in skeletal muscle of mice. Tables 1, 2, 15 and 16 describe aging-related increases and decreases in gene expression in gastrocnemius of mice. (Tables 1 and 2 were prepared using a high density oligonucleotide array of over 6,300 genes, while Tables 15 and 16 were prepared using a high density oligonucleotide array of 19,000 genes.) Tables 3 and 4 describe caloric restriction related decreases and increases in gene expression. Tables 1 and 2 contain a column ("CR reversal") describing the influence of caloric restriction on the increased or decreased expression. Tables 5-8 describe a similar analysis of the gene expression profile determined neocortex tissue of mice and Tables 9 and 10 describe a gene expression profile determined on the cerebellum tissue in mice. Tables 11-14 describe gene expression profiles determined in mouse heart. (Tables 11 and 12 were prepared with the 19,000 high density oligonucleotide chip, while Tables 13 and 14 were prepared using the less dense gene chip.) From these gene expression profiles, one may select many biomarkers.

For example, in order to either measure or determine biological age in skeletal muscle, one would select markers in Tables 1 and 2 that reflect changes in gene expression that have been shown to be either partially or completely inhibited by caloric restriction in skeletal muscle such as AA0071777, L06444, AA114576, etc. Genes that were not affected by caloric restriction (such as W84988, Table 1) may represent chronological markers or aging, and therefore are less useful for the measurement of aging rates. One

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may determine which genes are or are not affected by caloric restriction by examination of the "CR reversal" lane of Tables 1 or 2.

If one wished to examine a tissue, organ or cell that is not represented in Tables 1-16, one would prepare samples and tabulate results from those samples as described below in the Examples. In this manner, one may examine any tissue, organ or cell for biological aging. Preferably, one would wish to examine a tissue selected from the group consisting of brain tissue, heart tissue, muscle tissue, skin, liver tissue, blood, lymphocytes, skeletal tissue and mucosa.

For example, choosing markers from Tables 1 and 2 to examine the efficacy of a test compound in aging prevention, one could design a PCR-based amplification strategy or a DNA microarray hybridization strategy to quantify the mRNA abundance for markers W08057, AA114576, 11071777, 11106112, D29016 and M16465 as a function of aging, using animals of several age groups, such as 6 months, 12 months, 18 months, 24 months and 30 months. (The marker designations refer to Gene Bank accession number entries.) A second set of animals would be given a test compound intended to slow the aging process at 10 months of age (middle age). Animals from the experimental group would be sacrificed or biopsied at the ages of 12 months, 18 months, 24 months and 30 months. If the test compound is successful, the normal aging-related alterations in expression of these particular markers will be prevented or attenuated.

One would follow the same protocol in using the other tables for marker selection. One would match the tissue to be analyzed with the appropriate table. For example, if one were analyzing muscle tissue, one might choose markers from Tables 1 and 2.

In another embodiment, the present invention is a method of obtaining and validating novel mammalian biomarkers of aging. Preferably, this method

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comprises the steps of comparing the gene expression profile from a young subject's organ, tissue or cells with samples from individuals that are both chronologically and biologically aged. This is followed by comparison of the gene expression profile of the chronologically and biologically aged individuals with that of individuals that display similar chronological ages, but a younger biological age, such as animals under caloric restriction. Gene expression alterations that are prevented or retarded by caloric restriction represent markers of biological age, as opposed to chronological age.

In one version of this embodiment, one would preferably use high density oligonucleotide arrays representing at least 5-10% of the subject's genes, as described in Lee, et al. at Science 285(5432):1390-1393, 1999 and Lee, et al., Nat. Genet. 25(3):294-297, 2000. (Both Lee, et al., supra, 1999 and Lee, et al., supra, 2000 are incorporated by reference as if fully set forth herein.)

For example, Lee, et al., supra, 1999 details the comparison between gastrocnemius muscle from 5 month (young) and 30 month (aged) mice, and 30 month mice under caloric restriction. Lee, et al., supra, 1999 disclose that of the 6500 genes surveyed in the oligonucleotide array, 58 (0.9%) displayed a greater than 2-fold increase in expression levels as a function of age and 55 (0.8%) displayed a greater than 2-fold decrease in expression. The most substantial expression change was for the mitochondrial sarcomeric creatine kinase (Mi-CK) gene (3.8-fold). Sequences that display a greater than three-fold alteration (increase or decrease) with aging, which are prevented or restricted by caloric restriction, such as W08057, AA114576, AA071777, AA106112, D29016, M16465, are likely to be particularly good aging biomarkers.

Lee, et al., supra, 2000 describes the comparison between cDNAs isolated from neocortex tissue for the same three groups of mice described

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above. Lee, et al., supra, 2000 disclose that of the 6347 genes surveyed, 63 (1%) displayed a greater than 1.7-fold increase in expression levels with aging in the neocortex, whereas 63 genes (1%) displayed a greater than 2.1-fold increase in expression in the cerebellum. Functional classes were assigned and regulatory mechanisms inferred for specific sets of alterations (see Tables 5-10). Of these, 20% (13/63), and 33% (17-51) could be assigned to an inflammatory response in the neocortex and cerebullum, respectively. Transcriptional alterations of several genes in this category were shared by the two brain regions, although fold-changes tended to be higher in the cerebellum, perhaps due to reduced tissue size and/or reduced heterogeneity at the cellular level. These transcriptional alterations include the microglial and macrophage migration factor *Mps1* and the *Cd40L* receptor, which is a mediator of the microglial activation pathway. Also induced was *Lysozyme C* and beta(2) microglobulin which are markers of inflammation in the human CNS. Interestingly, a concerted induction of the complement cascade components *C4*, *C1qA*, *C1qB* and *C1qC* was observed, a part of the humoral immune system involved in inflammation and cytolysis.

In another embodiment, the present invention is a method of screening a test compound for the ability to inhibit or retard the aging process in mammalian tissue. In a typical example of this embodiment, one would first treat a test mammal with a test compound and then analyze a representative tissue of the mammal for the level of expression of a panel of biomarkers. Preferably, the tissue is selected from the group consisting of brain tissue, heart tissue, muscle tissue, blood, skeletal muscle, mucosa, skin and liver tissue. One then compares the analysis of the tissue with a control, untreated mammal and identifies test compounds that are capable of modifying the expression of the biomarker sequences in the mammalian samples such that the expression is indicative of tissue that has an inhibited or retarded

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biological age. This expression pattern would be more similar to an expression pattern found in biologically younger subjects.

As an example, a group of young rodents (mice) would be divided into a control and a test group. The test group would receive a test compound as a dietary supplement added to food from age 5 months to 30 months, whereas the control group would receive a standard diet during this time period. At age 30 months, several tissues would be collected from animals from each group, and a gene expression profile would be obtained. Each animal's gene expression profile would be compared to that of a 5 month (young) animals receiving the standard diet. One would then examine if, for any of the organs investigated, the gene expression pattern fo the animals receiving the test compound was more similar to that of young animals, compared to the experimental group that received a standard diet.

In another embodiment, the present invention is a method of detecting whether a test compound mimics the gene profile induced by caloric restriction. This method typically comprises the steps of exposing the mammal to a test compound and measuring the level of a panel of biomarkers. One then determines whether the expression pattern of the tissue mimics the expression pattern induced by caloric restriction.

For example, if one wished to examine skeletal muscle, the test compound would be analyzed for induction of genes observed to be induced by caloric restriction in Tables 3 and 4.

EXAMPLES

1. <u>In General</u>

In order to test our hypothesis, we performed gene expression profiling of over 6300 genes in skeletal muscle, neocortex tissue, and cerebellum tissue and 19,000 genes in skeletal muscle and heart tissue of 5-month and

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30-month old C57Bl6 mice, using high density oligonucleotide arrays. We found that a number of genes demonstrated alterations in gene expression profile as a function of chronological age and that these genes were broadly divided into a few classes listed in the Tables, such as stress response, energy metabolism, biosynthesis, protein metabolism and neuronal growth.

In order to validate the use of gene expression profiles as biomarkers of biological age, we investigated the role of caloric restriction, the only intervention known to retard the aging process in mammals, on gene expression profiles. Our analysis demonstrated that 30-month old calorically restricted animals display either complete or partial prevention of most aging associated alterations in gene expression, validating the use of gene expression profiles as a biomarkers of the aging process. In the process we have discovered a gene expression profile that is specifically associated with caloric restriction. We believe that this profile provides genetic markers for this metabolic state.

In like fashion, the present invention allows the determination of biological age in any organism through the determination of age-related variations in mRNA abundance. Such determination can be achieved through generation of cDNA from the mRNA of the organism and quantification of the cDNA product through hybridization to DNA microarrays, preferably as described here. Alternatively, any technique that allows for the quantitative determination of mRNA abundance may be used, such as quantitative PCR, Northern blotting and RNAse protection assays.

2. Experimental Protocols

Details on the methods employed to house and feed male C57BL/6 mice, a commonly used model in aging research with an average lifespan of ~30 months, were recently described (T.D. Pugh, et al., Cancer Res. 59:642, 1999). Briefly, mice were purchased from Charles River Laboratories

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(Wilmington, MA) at 1.5 months of age. After receipt in Madison, the mice were housed singly in the specific pathogen-free Shared Aging Rodent Facility at the Madison Veterans Administration Geriatric Research, Education and Clinical Center, and provided a non-purified diet (PLI5001 (Purina Labs, St. Louis, MO) and acidified water *ad libitum* for one week. The mice were then allocated into two groups and fed one of two nearly isocaloric (~4.1 kcal/g), semi-purified diets. Each mouse in the control group was fed 84 kcal/week of the control diet (TD91349 (Teklad, Madison, WI)) which is ~5-20% less than the range of individual *ad libitum* intakes. This dietary intake was used so that the control mice were not obese and retained motor activity up to the age of sacrifice. Each mouse subjected to CR was fed 62 kcal/week of the restricted diet (TD9351(Teklad, Madison, WI)), resulting in a 26% reduction of caloric intake. The latter diet was enriched in protein, vitamins and minerals such that caloric restriction (CR) and control mice were fed nearly identical amounts of these components. The fat component, corn oil, was at the same level (13.5%) in both diets, leading to a 26% reduction in fat intake for the calorie-restricted mice. The adult body weights of the mice averaged ~32 g for controls and ~23 g for those on CR. Mice were euthanized by rapid cervical dislocation, autopsied to exclude animals showing overt disease, and the gastrocnemius muscle was removed from each limb, combined in a micocentrifuge tube, and immediately flash-frozen in liquid nitrogen and then stored at -80°C. All aspects of animal care were approved by the appropriate committees and conformed with institutional guidelines.

Total RNA was extracted from frozen tissue using TRIZOL reagent (Life Technologies) and a power homogenizer (Fisher Scientific) with the addition of chloroform for the phase separation before isopropyl alcohol precipitation of total RNA. Poly(A)$^+$ RNA was purified from the total RNA with

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oligo-dT linked Oligotex resin (Qiagen). One microgram of poly(A)$^+$ RNA was converted into double-stranded cDNA (ds-cDNA) using SuperScript Choice System (Life Technologies) with an oligo dT primer containing a T7 RNA polymerase promoter region (Genset). After second strand synthesis, the reaction mixture was extracted with phenol/chloroform/isoamyl alcohol. Phase Lock Gel (5 Prime - 3 Prime, Inc.) was used to increase ds-cDNA recovery. The ds-cDNA was collected by ethanol precipitation. The pellet was resuspended in 3 µl of DEPC-treated water. *In vitro* transcription was performed using a T7 Megascript Kit (Ambion) with 1.5 µl of ds-cDNA template in the presence of a mixture of unlabeled ATP, CTP, GTP, and UTP and biotin-labeled CTP and UTP (bio-11-CTP and bio-16-UTP (Enzo)). Biotin-labeled cRNA was purified using a RNeasy affinity column (Quiagen). The amount of biotin-labeled cRNA was determined by measuring absorbance at 260 nm. Biotin-labeled cRNA was fragmented randomly to sizes ranging from 35 to 200 bases by incubating at 94°C for 35 minutes in 40 mM Tris-acetate pH 8.1, 100 mM potassium acetate, and 30 mM magnesium acetate. The hybridization solutions contained 100 mM MES, 1 M (Na^+), 20 mM EDTA, and 0.1% Tween 20. In addition, the hybridization solutions contained 50 pM oligonucleotide B2 (a biotin-labeled control oligonucleotide used for making grid alignments), 0.1 mg/mL herring sperm DNA, and 0.5 mg/mL acetylated BSA. The final concentration of fragmented cRNA was 0.05 µg/µl in the hybridization solutions. Hybridization solutions were heated to 99°C for 5 minutes followed by 45°C for 5 minutes before being placed in the gene chip. 10 µg of cRNA was placed in the gene chip. Hybridizations were carried out at 45°C for 16 hours with mixing on a rotisserie at 60 rpm. Following hybridization, the hybridization solutions were removed, and the gene chips were installed in fluidics systems for wash and stain. The fluidics system (Affymetrix GeneChip Fluidics tation 400)

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performed two post-hybridization washes (a non-stringent wash and a stringent wash), staining with streptavidin-phycoerythrin, and one post-stain wash. The gene chips were read at a resolution of 6 μm using a Hewlett Packard Gene array scanner. Data collected from two scanned images were used for the analysis.

Detailed protocols for data analysis of Affymetrix microarrays and extensive documentation of the sensitivity and quantitative aspects of the method have been described (D.J. Lockhart, Nature Biotech. 14:1675, 1996). The Affymetrix GeneChip MU6500 set was derived from selected genes and ESTs from the August 15, 1996 release of GeneBank. Briefly, each gene is represented by the use of ~20 perfectly matched (PM) and mismatched (MM) control probes. The MM probes act as specificity controls that allow the direct subtraction of both background and cross-hybridization signals. The number of instances in which the PM hybridization signal is larger than the MM signal is computed along with the average of the logarithm of the PM:MM ratio (after background subtraction) for each probe set. These values are used to make a matrix-based decision concerning the presence or absence of an RNA molecule. All calculations are performed by Affymetrix software. To determine the quantitative RNA abundance, the average of the differences representing PM minus MM for each gene-specific probe family is calculated, after discarding the maximum, the minimum, and any outliers beyond three standard deviations. For example, to calculate fold changes (FC) between data sets obtained from young (y) vs. old (o) mice, the following formula was used:

$$FC = \frac{SI_o - SI_y}{\text{the smallest of either } SI_y \text{ or } SI_o} + 1 \text{ if } SI_o \geq SI_o \text{ or } -1 \text{ if } SI_o < SI_y$$

Where SI_o is the average signal intensity from a gene-specific probe family from an old mouse and SI_y is that from a young mouse.

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Alternatively, if the Q_{factor}, a measure of the non-specific fluorescence intensity background, is larger the smallest of either SI_y or SI_o, the FC is calculated as:

$$FC = \frac{SI_o - SI_y}{Q_{factor}}$$

The Q_{factor} is automatically calculated for different regions of the microarray, and therefore minimizes the calculation of spurious fold changes. Average of pair-wise comparisons were made between study groups, each composed of three animals using Excel software. As an example, each 5-month-old mouse was compared to each 30-month-old mouse generating a total of nine pair-wise comparisons.

The murine 19K gene chip allows one to monitor more than 19,000 clustered murine EST transcripts selected from the TIGR (The Institute for Genome Research) database. This database is created by assembling ESTs into virtual transcripts called tentative mouse consensus sequences (Tcs). These sequence contigs are assigned a TC (tentative mouse consensus) number. Therefore, each TC number represents a unique transcript and allows one to check or obtain the sequence from the TIGR mouse gene index.

3. Results

The results of our analysis are shown below in Tables 1-16. Tables 1-4 and 15-16 are the result of the analysis of mouse gastrocnemias muscle. Tables 1 and 15 describe aging-related increases in gene expression, Tables 2 and 16 describe aging-related decrease in gene expression, Table 3 describes caloric restriction related increases, and Table 4 describes caloric restriction related decreases in gene expression. Tables 5-10 describe results obtained using mouse brain tissue. Table 5 describes aging-related increases in gene expression in neocortex, Table 6 describes aging-related

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decreases in gene expression in neocortex, Table 7 describes caloric restriction related increases in gene expression in neocortex, Table 8 describes caloric restriction related decreases in gene expression in neocortex, Table 9 describes aging-related increases in gene expression in the cerebellum, and Table 10 describes aging-related decreases in gene expression in the cerebellum.

Tables 11-14 are the result of the analysis of mouse heart muscle. Tables 11 and 12, obtained by use of the Mu19K Gene Chip, disclose up-regulated and down-regulated aging-related genes. Tables 13 and 14, obtained from the Mu6500 Gene Chip, disclose up-regulated and down-regulated aging-related genes.

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Table 1. Aging-related increases in gene expression in gastrocnemius muscle of C57BL/6 mice

ORF	Δ Age (fold)	Gene	Class/Function	CR Reversal
AA106112	3.8	Mitochondrial Sarcomeric Creatine Kinase	Energy Metabolism/ATP generation	C
AA071777	3.8	Synaptic Vesicle Protein 2	Growth Factor/Neurite extension	51%
Y00094	3.6	Ypt 1/ras-related GTP Binding Protein	Transport/Protein trafficking	C
W10855	3.5	Methyl CpG Binding Protein	DNA metabolism/gene silencing	C
W08057	3.5	Heat Shock 27 kDa Protein	Stress Response/Chaperone	C
M17790	3.5	Serum Amyloid A Isoform 4	Stress Response/Unknown	N
L06444	3.5	GDF-9	Growth Factor/Unknown	50%
AA114576	3.4	Heat Shock 71 kDa Protein	Stress Response/Chaperone	C
W84988	3.3	Transcription Regulatory Protein SWI3	Transcriptional Factor/Unknown	N
X64587	3.2	U2AF	RNA Metabolism/Splicing Factor	C
D87902	3.2	ARF5	Transport/ADP-ribosylation	87%
U19118	3.0	LRG-21	Transcriptional Factor/Macrophage activation	42%
AA068057	2.9	RabB	Signal Transduction/Unknown	C
U05837	2.9	Beta-Hexosaminidase	Catabolism/Lysosomal enzyme	C
W85446	2.8	Protein Kinase C Inhibitor 1 Homolog	Signal Transduction/Unknown	74%
AA060167	2.8	Pre-B Cell Enhancing Factor Precursor	Growth Factor/Cytokine	C
M37760	2.7	Serine-2 Ultrahigh Sulfur Protein	Unknown	45%
AA096992	2.7	G25K GTP-Binding Protein	Signal Transduction/Unknown	N
AA008255	2.7	Adaptin Complex Small Chain Homolog	Unknown	37%
AA166502	2.6	EIF-4A-II	RNA Metabolism/RNA helicase	N
X66602	2.6	POU-domain protein	Transcriptional Factor/Unknown	N
X79828	2.6	NK 10	Transcriptional Factor/Unknown	N
V00719	2.6	Alpha-Amylase-1	Energy Metabolism/Starch metabolism	N
L28177	2.6	GADD45	Stress Response/Cell cycle checkpoint	77%
W50941	2.5	Nucleotide Pyrophosphatase	Unknown	N
X53257	2.5	Neurotrophin-3	Growth Factor/Reinnervation of muscle	50%
M74570	2.4	Aldehyde Dehydrogenase II	Stress Response/Aldehyde detoxification	29%
D49473	2.4	Sox17	Transcriptional Factor/Unknown	86%
AA117284	2.3	Zinc Finger Protein 43 (HTF6)	Transcriptional Factor/Unknown	N
W63835	2.3	Beta-centractin	Structural/Contractility	60%
AA089097	2.2	Phosphatidylcholine-transfer Protein	Transport/Lipid turnover	C
AA059662	2.2	Protease Do Precursor	Stress Response/Protease	C
L22482	2.2	HIC-5	Stress Response/Senescence and differentiation	C
X78197	2.2	AP-2 Beta	Transcriptional Factor/Neurogenesis	N
AA059664	2.2	IGF Binding Protein	Growth Factor/Cellular senescence	C
V00714	2.2	Alpha Globin	Structural/Hemoglobin component	C
X99963	2.2	rhoB	Stress Response/Unknown	87%
AA014024	2.1	Dynactin	Transport/Neuronal transport	55%
X65627	2.1	TNZ2	Stress Response/RNA metabolism	64%
X95503	2.1	GTP-Binding Protein (IRG-47)	Signal Transduction/Unknown	85%
V00727	2.1	FBJ-MuSV	Provirus/None	C
X12807	2.1	pp2.5	Unknown	C
W08049	2.1	MAGP	Structural/Microfibril glycoprotein	N
AA066425	2.1	CO-029	Structural/Cell surface glycoprotein	N
W82998	2.1	POLYA+ RNA Export Protein	RNA Metabolism/RNA export	44%
X89749	2.1	mTGIF	Transcriptional Factor/Neuronal differentiation	C
L07918	2.1	GDP-Dissociation Inhibitor	Transport/membrane dynamics	N
X63190	2.1	PEA3	Transcriptional Factor/Response to muscle injury	C

*The influence of CR on the increased expression with age of specific ORFs is denoted as either C (complete, ≥90%), N (none) or partial (≥20%, percentage effect indicated).

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Table 2. Aging-related decreases in gene expression in gastrocnemius muscle of C57BL/6 mice*

ORF	Δ Age (fold)	Gene	Class/Function	CR Reversal
D29016	-6.4	Squalene Synthase	Biosynthesis/Cholesterol/fatty acid synthesis	52%
AA106126	-4.9	Myosin Heavy Chain, Perinatal	Structural Protein/Muscle contraction	C
D31898	-4.4	Protein Tyrosine Phosphatase, PTPBR7	Signal Transduction/Unknown	79%
U29762	-4.3	Albumin Gene D-Box Binding Protein	Transcriptional Factor/Albumin synthesis	85%
AA061310	-4.1	Mitochondrial LON Protease	Energy Metabolism:Mitochondrial biogenesis	C
AA162443	-3.6	Protein Phosphatase PP2a	Signal Transduction/Unknown	C
M89797	-3.5	Wnt-4	Signal Transduction/Unknown	72%
M16465	-3.4	Calpactin I Light Chain	Signal Transduction/Calcium effector	C
X74134	-3.2	Ovalbumin Transcription Factor I	Transcriptional Factor/Unknown	N
U08020	-3.2	Alpha 1 Type 1 Collagen	Structural Protein/Extracellular matrix	N
X58251	-3.1	Pro-alpha-2(I) Collagen	Structural Protein/Extracellular matrix	N
AA138226	-3.1	Clathrin Light Chain B	Intracellular Transport/Vesicle transport	C
X85214	-3.0	Ox40	Signal Transduction/T Cell activation	50%
D76440	-2.9	Necdin	Growth Factor/neuronal growth suppressor	47%
AA107752	-2.9	EF-1-Gamma	Protein Metabolism:Protein synthesis	63%
W55037	-2.9	Alpha Enolase	Energy Metabolism:Glycolysis	68%
X74134	-2.8	COUP-TFI	Transcription Factor/Unknown	28%
U06146	-2.8	Desintegrin-related Protein	Unknown	28%
U39545	-2.8	BMP8b	Growth Factor/Unknown	C
X75014	-2.7	Phox2 Homeodomain Protein	Transcriptional Factor/Neuronal differentiation and survival	65%
U22031	-2.6	20S Proteasome Subunit	Protein Metabolism:Protein turnover	44%
U70210	-2.5	TR2L	Transcriptional Factor/Apoptosis modulator	N
X76652	-2.5	3f8	Structural Protein/Neuronal adhesion	N
W54288	-2.5	PKCSH	Signal Transduction/Unknown	C
M81475	-2.5	Phosphoprotein Phosphatase	Energy Metabolism:Glycogen metabolism	C
U22394	-2.3	mSin3	Transcriptional Factor/Inhibitor of cell proliferation	46%
M83336	-2.3	gp130	Signal Transduction/Unknown	77%
L34611	-2.3	PTHR	Signal Transduction/Ca homeostasis	N
X52046	-2.3	Pro-Alpha1 (III) Collagen	Structural Protein//Extracellular matrix	N
L2450	-2.2	DNA Binding-protein	Unknown	58%
AA103356	-2.2	Calmodulin	Signal Transduction/Calcium effector	N
L37092	-2.2	p130PITSL Cyclin-kinase	DNA Metabolism/Cell cycle control	N
AA061604	-2.2	Ubiquitin Thiolesterase	Protein Metabolism:Protein turnover	C
AA139680	-2.2	DNA Polymerase Alpha Primase	DNA Metabolism/DNA replication	N
AA034842	-2.1	ERV1	DNA Metabolism/Maintenance of MtDNA	46%
M21285	-2.1	Stearoyl-CoA Desaturase	Biosynthesis/PUFA synthesis	C
U11274	-2.1	PmuAUF1-3	RNA Metabolism/RNA degradation	N
U73744	-2.1	HSP70	Stress Response/Chaperone	N
J03398	-2.1	MDR	Membrane Protein/Unknown	N
AA145829	-2.1	26S Proteasome Component TBP1	Protein Metabolism:Protein turnover	C
M32240	-2.1	GAS3	Growth Factor/Apoptosis and growth arrest	55%
L00681	-2.1	Unp Ubiquitin Specific Protease	Protein Metabolism:Protein turnover	N
U34277	-2.0	PAF Acetylhydrolase	Unknown	N
U35741	-2.0	Rhodanese	Protein Metabolism:Mitochondrial protein folding	C
W53731	-2.0	Signal Recognition Particle Receptor	Intracellular Transport/Protein trafficking	C
AA044497	-2.0	Zinc Finger Protein 32	Transcriptional Factor/Unknown	40%
L27842	-2.0	PMP35	Energy Metabolism:Peroxisome assembly	60%
AA106406	-2.0	ATP Synthase A Chain	Energy Metabolism:ATP synthesis	N
AA041826	-2.0	IPP-2	Energy Metabolism:Glycogen Metabolism	C

*The influence of CR on the increased expression with age of specific ORFs is denoted as either C (complete, ≥90%), N (none) or partial (≥20%, percentage effect indicated).

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Table 3. Caloric restriction-related increases in gene expression*

ORF	↑ CR (fold)	Gene	Class/Function
U68267	9.6	Myosin Binding Protein H (MyBP-H)	Structural/Myofibril interactions
X13135	4.7	Fatty Acid Synthase	Biosynthesis/Fatty acid synthesis
U05809	4.5	LAF1 Transketolase	Energy Metabolism/Carbohydrate metabolism
W53351	4.1	Fructose-bisphosphate Aldolase	Energy Metabolism/Glycolysis
M15501	3.5	Cardiac Muscle Alpha Actin	Structural/Muscle contraction
AA071776	3.5	Glucose-6-Phosphate Isomerase	Energy Metabolism/Glycolysis
AA073283	3.3	Cardiac Muscle Myosin Beta-Actin	Structural/Contractile protein
AA138226	2.9	Clathrin Light Chain B	Transport/Axonal transport
L42115	2.9	Insulin-Activated Amino Acid Transporter	Transport/Aminoacid transport
U37222	2.8	Adipocyte Complement-Related Protein (Acrp30)	Growth Factor/Unknown
W89939	2.7	FK506-Binding Protein (FKBP-12)	Signal Transduction/Neuronal regeneration
X16314	2.5	Glutamine Synthetase	Biosynthesis/Glutamine synthesis
AA080277	2.5	Sodium Potassium ATPase Alpha-2 Chain	Membrane Protein/Ion pump
W30250	2.5	Myosin Light Chain 1	Structural/Contractile protein
AA137659	2.4	Cytochrome P450-IIC12	Biosynthesis/Steroid biosynthesis
AA031112	2.4	ZFP-37	Transcriptional Factor/Unknown
U34295	2.3	Glucose Dependent Insulinotropic Polypeptide	Energy Metabolism/Insulin sensitizer
W54288	2.3	Protein Kinase-C Substrate (80K-H)	Signal Transduction/AGE receptor
U01841	2.3	Peroxisome Proliferator Receptor Gamma (PPAR)	Energy Metabolism/Insulin sensitizer
AA109527	2.3	Actin 1	Structural/Contractile protein
AA145829	2.3	26S Protease Subunit TBP-1	Protein Metabolism/26S proteasome component
Y00137	2.3	Lymphotoxin-Beta	Signal Transduction/Cytokine
AA107752	2.2	Elongation Factor 1-gamma	Protein Metabolism/Protein synthesis
AA016431	2.2	Keratinocyte Lipid-binding Protein	Unknown/Fatty acid binding
M93275	2.1	Adipose Differentiation Related Protein (ADFP)	Unknown
W53731	2.1	Signal Recognition Particle Receptor Alpha Subunit	Protein Metabolism/Protein synthesis
U60328	2.1	Proteasome Activator PA28 Alpha Subunit	Protein Metabolism/Protein turnover
W78478	2.1	Gamma E-crystallin	Unknown
X67083	2.1	Chop-10 (gadd153)	Stress-Response/Growth arrest
U40189	2.1	Neuropeptide Y	Unknown
AA020281	2.1	Progesterone Reductase	Metabolic/Progesterone metabolism
AA022083	2.0	Huntingtin	Unknown
X59990	2.0	mCyP-S1 (Cyclophilin)	Protein Metabolism/Protein folding
X56548	2.0	Purine Nucleoside Phosphorylase	Biosynthesis/Purine turnover
L28116	2.0	PPAR Delta	Energy Metabolism/Peroxisome induction
U43319	2.0	Frizzled 6	Unknown
X14432	2.0	Thrombomodulin	Unknown
L32973	2.0	Thymidylate Kinase	Biosynthesis/dTTP sythesis
D76440	1.9	Necdin	Growth Factor/Neuronal growth suppressor
L36860	1.9	GCAP	Signal Transduction/Calcium-binding regulatory protein
W08293	1.9	Translocon-Associated Protein Delta	Protein Metabolism/Protein translocation
AA041826	1.9	Protein Phosphatase Inhibitor 2 (IPP-2)	Energy Metabolism/Inhibition of glycogen synthesis
D42083	1.9	Fructose 1,6-bisphosphatase	Energy Metabolism/Gluconeogenesis
AA008737	1.9	Peroxisomal Protein PAS8	Transport/Peroxisome targeting
W57495	1.8	60S Ribosomal Protein L23	Protein Metabolism/Protein synthesis
D83585	1.8	Proteasome Z Subunit	Protein Metabolism/Protein turnover
M13366	1.8	Glycerophosphate Dehydrogenase	Energy Metabolism/Electron transport to mitochondria
U37091	1.8	Carbonic Anhydrase IV	Energy Metabolism/CO_2 disposal

*The genes listed on this table were not influenced by age. Reversal of aging-associated changes are listed in Tables 1 and 2. Energy Metabolism and Biosynthetic classes are highlighted in blue.

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Table 4 Caloric restriction-related decreases in gene expression

ORF	Δ DR (fold)	Gene	Class/Function
AA062328	-3.4	DnaJ Homolog 2	Stress Response Chaperone
X03690	-2.5	Ig Heavy Chain Constant Region mu(b)	Immune Function/Antibody
U60453	-2.3	Ezh1 (Zeste Homolog 2)	Transcriptional Factor/Gene silencing
M83380	-2.3	relB	Transcriptional Factor/Unknown
D38613	-2.1	921-L Presynaptic Protein	Unknown
X82457	-2.0	es64	Unknown
U35646	-2.0	Aminopeptidase	Protein Metabolism/Protein turnover
W13412	-1.9	ATP Synthase Coupling Factor B	Energy Metabolism/ATP synthesis
M92416	-1.9	FGF-6	Growth Factor/Muscle regeneration
U58497	-1.9	mp86 (Mnb Protein Kinase)	Signal Transduction/Unknown
L29454	-1.9	Fbn-1 (Fibrillin)	Structural/Microfibril organization
U56773	-1.9	Pelle-like Protein Kinase	Signal Transduction/Unknown
D49439	-1.9	TFIID Subunit p80	Transcriptional Factor/Unknown
D31943	-1.9	Inducible SH2-Containing Protein	Growth Factor/Cytokine
U47737	-1.9	TSA-1	Signal Transduction/T cell function
X63023	-1.9	Cytochrome P-450-IIIA	Stress Response Detoxification
X53476	-1.8	HMG-14	DNA Metabolism Chromatin remodeling
L33768	-1.8	JAK3	Signal Transduction/T cell function
U03283	-1.8	Cyp1b1 Cytochrome P450	Stress Response Detoxification
U14390	-1.8	Aldehyde Dehydrogenase-3	Stress Response Detoxification
U75530	-1.8	PHAS-II	Protein Metabolism/Translation inhibitor
X13605	-1.8	Histone H3.3	DNA metabolism/Chromatin remodeling
U65313	-1.8	G3BP	DNA metabolism/Helicase
AA062349	-1.8	P31	Protein Metabolism/Protein turnover
X76850	-1.8	MAPKAP2	Stress Response/Unknown
D43694	-1.8	Math-1	Transcription Factor/Neuronal differentiation
U66887	-1.8	RAD50	DNA Metabolism/DNA repair
M83219	-1.8	MRP14	Growth Factor/Inflammation
Z14986	-1.8	SAMDC	Biosynthesis/Polyamine synthesis
W17516	-1.8	NEDD8	Unknown
D78641	-1.7	Membrane Glycoprotein	Unknown
D26123	-1.7	Carbonyl Reductase	Unknown
U71205	-1.7	nit	Signal Transduction/Unknown
U31510	-1.7	ADP-ribosyltransferase	Protein Metabolism/ADP-ribosylation
L4406	-1.7	Hsp105-beta	Stress Response Chaperone
AA059718	-1.7	DNA Polymerase Beta	DNA Metabolism DNA repair
D16464	-1.7	HES-1	Transcription Factor/Neuronal differentiation
D87963	-1.7	ETFR-1	Transcriptional Factor/Unknown
U12236	-1.7	Alpha M290 Integrin	Signal Transduction/Cell and matrix adhesion
X98848	-1.7	6-phosphofructo-2-kinase	Energy Metabolism/glycolysis
W41974	-1.7	ATP-Dependent RNA Helicase-Homolog	RNA Metabolism/Unknown
X75285	-1.6	Fibulin-2	Structural/Basement membrane
M96265	-1.6	GALT	Energy Metabolism/Glycolysis
D67015	-1.6	97kDa Nuclear Pore Targeting Complex	Transport/Nuclear import
AA002750	-1.6	5-lipoxygenase Activating Protein (FLAP)	Biosynthesis/Leukotriene synthesis
X93357	-1.6	SYT	Transcriptional Factor/Unknown
W13191	-1.6	Thyroid Hormone Receptor Alpha-2	Metabolic/Thyroid hormone receptor
U43206	-1.6	Phosphatidylethanolamine Binding Protein	Signal Transduction/Unknown
W11169	-1.6	SUI1ISO1	Protein Metabolism/Translation initiation factor
W42234	-1.6	XPE	DNA Metabolism DNA repair
W08897	-1.6	Seryl-tRNA Synthetase	Protein Metabolism/Protein synthesis
AA027739	-1.6	Heterogeneous Nuclear Ribonucleoprotein K	Transcriptional Factor/Unknown

*The genes listed on this table were not influenced by age. Reversal of aging-associated changes are listed in Tables 1 and 2. DNA Repair and Stress Response classes are highlighted in green.

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Table 5. Aging-related increases in gene expression in neocortex of C57BL/6 mice*

ORF	Δ Age (fold)	SE	Signal Intensity		Gene	Class	CR Prevention
			Old	Young			
M88354	5.7	1.9	165	-109	Vasopressin-neurophysin II	Osmotic stress	68%
M17440	4.9	0.2	786	141	Complement C4	Immune/inflammatory	52%
AA120109	4.1	0.8	278	65	Interferon-induced protein 6-16 homolog	Immune/inflammatory	100%
M88355	2.7	0.6	195	70	Oxytocin-neurophysin	Osmotic stress	23%
AA037945	2.5	0.2	254	73	Beta-SNAP homolog	Transport	N
AA162093	2.5	0.2	145	21	Pre-mRNA splicing factor PRP22	RNA metabolism	N
AA137962	2.4	0.2	150	39	RAS-related protein RAB-14	Neurotransmitter release	N
K01347	2.3	0.4	420	178	Glial fibrilary acidic protein (GFAP)	Stress response	38%
AA027404	2.3	0.1	129	-43	Na/K-transporting ATPase beta-2 chain	Ionic transport	N
U60593	2.3	0.4	279	131	Cap43	Stress response	N
AA137871	2.3	0.6	55	-35	Phosphatidylinositol-4-phosphate 5-kinase	Signal transduction	N
U61751	2.3	0.2	299	128	VAMP-1	Transport	N
M21050	2.2	0.2	209	74	Lysozyme C	Immune/inflammatory	54%
AA153990	2.2	0.9	343	155	GTP:AMP phosphotransferase mitochondrial	Energy metabolism	100%
W29462	2.1	0.3	114	-49	Calpactin I light chain	Structural	N
L39123	2.1	0.2	1887	768	Apolipoprotein D (apoD)	Stress response	N
U16297	2.0	0.5	124	47	Cytochrome B561	Transport	N
M26251	2.0	0.3	484	260	Vimentin	Stress response	N
AA163911	2.0	0.2	130	38	Casein kinase I, delta isoform	Stress response	N
AA022006	2.0	0.2	115	-48	CD40L receptor precursor	Immune/inflammatory	N
AA124859	2.0	0.2	17	-54	ICAM-2	Immune/inflammatory	N
Y00305	1.9	0.2	225	101	Potassium channel protein-1	Transport	N
AA116604	1.9	0.1	515	272	Cathepsin Z	Stress response	70%
M95200	1.9	0.3	168	92	Vascular endothelial growth factor	Growth factor	N
L16894	1.9	0.4	123	-71	Cyclophilin C-AP	Stress response	100%
L20315	1.9	0.2	120	66	MPS1 gene	Immune/inflammatory	N
AA028501	1.9	0.2	74	16	Cytochrome c oxidase subunit VIII-H	Energy metabolism	N
X86569	1.9	0.2	24	-31	LIM-kinase	Unknown	N
AA105716	1.9	0.2	107	14	Fructose-1,6-bisphosphatase homolog	Energy metabolism	87%
W13646	1.8	0.1	1278	705	TI-225 (ubiquitin)	Stress response	N
J03236	1.8	0.3	681	362	JunB	Stress response	46%
X52886	1.8	0.1	1050	555	Cathepsin D	Stress response	64%
AA028273	1.8	0.3	331	153	Protein phosphatase inhibitor 2 (IPP-2)	Unknown	N
X16995	1.8	0.1	757	375	N10	Steroid metabolism	N
X16995	1.8	0.1	624	363	Complement C1q B-chain	Immune/inflammatory	100%
X66295	1.8	0.1	823	467	Complement C1q C-chain	Immune/inflammatory	75%
U22445	1.8	0.5	201	160	Serine/threonine kinase (Akt2)	Energy metabolism	100%
U17297	1.8	0.2	6	-43	Integral membrane phosphoprotein 7.2b	Unknown	N
AA059700	1.8	0.2	1467	797	MHC class I B(2)-microglobulin	Immune/inflammatory	64%
L29503	1.8	0.1	192	103	Myelin/oligodendrocyte glycoprotein (Omg)	Unknown	N
AA168918	1.8	0.4	326	166	Na/K-transporting ATPase gamma chain	Transport	N
M90364	1.8	0.1	326	202	Beta-catenin	Stress response	N
AA061086	1.8	0.2	179	89	Hsp40	Stress response	52%
W50891	1.8	0.3	41	-3	Creatine kinase	Energy metabolism	N
W67046	1.8	0.2	105	71	Exodus-2	Immune/inflammatory	N
W13875	1.8	0.2	216	125	Myosin regulatory light chain 2-A	Unknown	N
X67083	1.8	0.3	121	47	Chop-10 GADD153	Stress response	N
AA089110	1.8	0.2	23	-35	Dynein beta chain, ciliary	Transport	N
V00727	1.7	0.3	404	236	c-fos(p55)	Stress response	100%
AA062328	1.7	0.2	113	23	DNAJ protein homolog 2	Stress response	N

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AA122619	1.7	0.3	14	-43	Set protein (HLA-DR associated protein II)	Unknown	N
M73741	1.7	0.2	1313	730	Alpha-B2-crystallin gene	Stress response	67%
X70393	1.7	0.4	146	65	Inter-alpha-inhibitor H3 chain	Immune/inflammatory	56%
AA124698	1.7	0.7	100	42	Lethal(1)discs large-1	Unknown	N
W14434	1.7	0.2	401	240	Fructose-bisphosphate aldolase	Energy metabolism	N
W89579	1.7	0.2	83	-3	RAS-related protein RAB-4	Signal transduction	N
AA089333	1.7	0.1	336	221	Cathepsin S precursor	Stress response	56%
U19521	1.7	0.2	70	31	Vesicle transport protein (munc-18c)	Transport	N
AA107137	1.7	0.3	204	118	Casein kinase I, gamma	Unknown	N
AA106166	1.7	0.2	2312	1372	Elongation factor 2 (EF-2) homolog	RNA metabolism	N
M31811	1.7	0.1	748	457	Clathrin light chain B	Transport	100%
AA140487	1.7	0.3	23	-25	Cyclophilin A homolog	Stress response	100%
U37419	1.7	0.2	58	-29	G protein alpha subunit (GNA-15)	Signal transduction	N
AA114781	1.7	0.2	52	26	Uridylate kinase	DNA metabolism	N
X58861	1.6	0.1	1128	694	Complement C1Q alpha-chain	Immune/inflammatory	100%
AA048650	1.6	0.2	169	100	Estradiol 17 B-dehydrogenase 3 homolog	Steroid metabolism	N
W46723	1.6	0.2	83	46	Creatine kinase, B chain homolog	Energy metabolism	N
U16162	1.6	0.7	112	82	Prolyl 4-hydroxylase alpha(I)-subunit	Structural	N
X68273	1.6	0.2	105	73	Macrosialin	Immune/inflammatory	N
W48952	1.6	0.7	87	38	B-adrenergic receptor kinase 1	Signal transduction	N
AA063858	1.6	0.2	135	80	RHO-related GTP-binding protein RHOG	Signal transduction	100%
M15525	1.6	0.1	22	-58	Laminin B1	Neuronal outgrowth	N
AA068780	1.6	0.1	275	187	Phosphoserine aminotransferase homolog	Unknown	76%
U27462	1.6	0.3	133	79	BS4 peptide	Unknown	N
AA106077	1.6	0.1	116	64	Glutathione peroxidase	Stress response	76%
AA119959	1.6	0.2	194	128	Protein transport protein SEC23	Transport	N
AA061170	1.6	0.2	39	-18	NEDD-4 protein	Unknown	N
X16151	1.6	0.2	93	61	T-lymphocyte activation 1 protein (ETa-1)	Immune/inflammatory	N
W29462	1.6	0.3	114	-49	Calpactin I light chain (p11)	Unknown	N
AA097579	1.6	0.1	24	-20	Zinc finger protein 91 homolog	Unknown	52%
X64070	1.6	0.3	252	163	46kDa mannose 6-phosphate receptor	Lysosomal	N
W48519	1.6	0.2	98	100	GRP94 homolog	Stress response	N
X78682	1.6	0.2	408	269	B-cell receptor associated protein (BAP) 32	Unknown	N
AA106166	1.6	0.2	2312	1372	Elongation factor 2 homolog	Protein metabolism	N
AA169054	1.6	0.2	279	184	GTP-binding protein GTR1	Signal transduction	N
W51181	1.6	0.3	42	25	DNA-directed RNA polymerase II	RNA metabolism	75%
AA036390	1.6	0.2	146	83	DNA-binding protein inhibitor ID-1	Transcriptional factor	75%
L08115	1.5	0.2	309	236	Human CD9 antigen homolog	Structural	100%
U37353	1.5	0.2	191	121	Protein phosphatase 2A B'alpha3 regulatory subunit	Signal transduction	N
L10244	1.5	0.2	316	206	Spermidine/spermine N1-acetyltransferase	Polyamine metabolism	N
J05154	1.5	0.2	72	6	Cholesterol acyltransferase (LCAT)	Steroid metabolism	N
D43643	1.5	0.2	62	36	YL-1	Unknown	N
M34141	1.5	0.1	39	5	COX-1	Immune/inflammatory	100%
L28177	1.5	0.1	35	-9	GADD 45	Stress response	N
X85992	1.5	0.1	51	10	Semaphorin C	Neuronal remodelling	N
AA098307	1.5	0.2	85	47	Tubulin beta 5	Microtubule component	N

*The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

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Table 6. Aging-related decreases in gene expression in neocortex of C57BL/6 mice*

ORF	Δ Age (fold)	SE	Signal Intensity		Gene	Class	CR Prevention
			Old	Young			
X74134	-3.0	1.1	157	387	Ovalbumin upstream promoter	Transcriptional factor	N
L24430	-2.7	0.6	56	161	Osteocalcin precursor	Unknown	N
AA124352	-2.5	0.5	19	274	Neuromedin B precursor homolog	Neurotransmission	54%
D31898	-2.2	0.5	116	253	Protein tyrosine phosphatase, PTPBR7	Unknown	N
W29468	-2.2	0.3	133	284	Myosin light chain 2 mRNA	Unknown	N
AA065993	-2.2	0.3	16	115	GTP-binding nuclear protein RAN homolog	Signal transduction	N
U35323	-2.1	0.3	11	135	H2-M	Unknown	N
W98695	-2.1	0.2	3	120	Plasma retinol-binding protein precursor	Steroid metabolism	N
AA062463	-2.1	0.2	63	168	Kidney androgen-regulated protein	Steroid metabolism	N
U38196	-2.1	0.6	64	151	Palmytoylated protein p55	Signal transduction	100%
L36135	-2.1	0.3	-42	32	T cell receptor delta chain, C region	Immune/inflammatory	N
D32200	-2.1	0.3	38	101	Hes-3	Unknown	N
W98898	-2.1	0.4	-21	125	Transforming protein RFP	Growth factor	N
U29762	-2.0	0.2	396	744	Albumin gene D-Box binding protein	Circadian rhythm	N
AA138711	-2.0	0.5	222	321	Protein kinase C inhibitor protein	Unknown	N
W13586	-2.0	0.3	135	548	Atrial/fetal isoform myosin alkali light chain	Structural	49%
X67812	-2.0	0.3	41	120	ret proto-oncogene	Unknown	N
M97812	-2.0	0.2	12	85	REX-1	Steroid metabolism	N
W11011	-2.0	0.4	418	673	NEDD8	Protein metabolism	N
X13538	-2.0	0.2	66	176	Hox-1.4 gene	Growth factor	N
X66405	-2.0	0.5	186	330	Collagen alpha 1 chain type VI	Structural	100%
AA050791	-2.0	0.5	194	355	Creatine kinase, M chain	Energy metabolism	N
W55515	-1.9	0.4	132	243	Cyclic-AMP-dependent ATF-4	Transcriptional factor	100%
L23416	-1.9	0.3	184	291	Clone p85 secreted protein	Unknown	100%
X70398	-1.9	0.9	186	325	PTZ-17	Growth factor	N
M84412	-1.8	0.1	46	128	Antigen (Ly-9)	Immune/inflammatory	47%
AA067927	-1.8	0.2	63	132	DNA-PK-catalytic subunit	DNA metabolism	N
Y09585	-1.8	0.4	143	212	Serotonin 4L receptor	Neurotransmission	N
X95255	-1.8	0.1	6	72	Gli3 protein	Growth factor	N
U37459	-1.8	0.1	37	87	Glial-derved neurotrophic factor (GDNF)	Growth factor	N
M99377	-1.8	0.3	121	270	Alpha-2 adrenergic receptor	Neurotransmission	N
D83585	-1.8	0.5	916	1457	Proteasome Z subunit	Protein metabolism	N
U52222	-1.8	0.2	61	160	Mel-1a melatonin receptor	Neuropeptide	N
M13710	-1.7	0.3	120	219	Interferon alpha-7 gene	Immune/inflammatory	N
D76446	-1.7	0.2	103	199	TAK1	Stress response	N
U64445	-1.7	0.2	12	56	Ubiquitin fusion-degradation protein (ufd1l)	Protein metabolism	100%
U39545	-1.7	0.3	144	235	Bone morphogenetic protein 8B (Bmp8b)	Growth factor	N
W59776	-1.7	0.2	95	174	Vacuolar ATP synthase catalytic subunit A	pH regulation	N
AA071792	-1.7	0.2	36	89	GSTP-1	Protein metabolism	N
AA052547	-1.7	0.3	-2	95	PA-FABP homolog	Unknown	100%
D63819	-1.7	0.2	61	143	Neuropeptide Y-Y1I receptor	Neuropeptide	N
W08326	-1.7	0.2	173	265	51PK(L) homolog	Unknown	N
AA000466	-1.7	0.2	113	195	p55CDC	DNA metabolism	100%
U66203	-1.7	0.2	111	181	FHF-3	Growth factor	N
AA051632	-1.7	0.2	112	167	MEK5	Signal transduction	61%
AA051147	-1.7	0.2	114	264	Chemotaxis protein cheY homolog	Unknown	N
X84692	-1.7	0.2	24	91	Spnr mRNA for RNA binding protein	RNA metabolism	N
U53925	-1.7	0.3	100	169	HCF1	Unknown	33%
AA038142	-1.7	0.3	251	376	RCC1	DNA metabolism	N

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W54682	-1.7	0.1	87	186	Antithrombin-III precursor (ATIII)	Immune/inflammatory	N
U13705	-1.7	0.2	324	494	Plama glutathione peroxidase (MUSPGPX)	Stress response	44%
X75384	-1.7	0.2	91	158	SAX-1	Growth factor	N
Z32767	-1.7	0.3	117	205	RAD52	DNA metabolism	76%
AA107752	-1.6	0.6	225	336	Elongation factor 1-gamma	Protein metabolism	N
M12836	-1.6	0.6	56	116	T-cell receptor gamma chain gene C-region	Immune/inflammatory	N
AA060704	-1.6	0.2	975	1407	Glutathione S-transferase MU 5	Unknown	N
AA118294	-1.6	0.1	99	161	Vitronectin homolog	Unknown	N
AA123026	-1.6	0.1	72	166	Pancreatitis-associated protein 3 homolog	Unknown	100%
AA065652	-1.6	0.1	39	99	Ubiquitin carboxyl-terminal hydrolase	Protein metabolism	N
W46104	-1.6	0.2	19	58	DNA-repair protein XP-E	DNA metabolism	N
M88694	-1.6	0.2	67	109	Thioether S-methyltransferase	Unknown	57%
AA117004	-1.6	0.1	6	61	Heat shock cognate 71 KD protein homolog	Stress response	N
M15501	-1.6	0.1	229	325	Adult cardiac muscle alpha-actin	Structural	100%
U49430	-1.6	0.2	78	108	Ceruloplasmin	Transport	N
X69019	-1.6	0.2	36	71	Hox 3.5 gene, complete cds	Growth factor	N
M28666	-1.6	0.2	317	496	Porphobilinogen deaminase	Biosynthesis	44%
W368759	-1.6	0.1	49	112	CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3- sialyltransferase	Sialytransferase	N
W11666	-1.6	0.2	105	207	apolipoprotein H	Lipid metabolism	N
W09925	-1.6	0.1	26	102	Endothelial actin-binding protein	Growth factor	74%
AA116282	-1.6	0.1	140	355	TNF alpha precursor	Immune/inflammatory	56%
D37791	-1.6	0.0	556	895	Beta-1,4,-galactosyltransferase	Unknown	N
W12658	-1.6	0.2	143	216	FKBP-rapamycin associated protein (FRAP)	Unknown	N
Z468454	-1.6	0.2	-16	39	Preproglucagon	Energy metabolism	N
AA103045	-1.5	0.1	57	106	Cleavage stimulation factor, 64 Kd subunit	RNA metabolism	N
AA108891	-1.5	0.2	4	62	Putative ATP-dependent RNA helicase	RNA metabolism	55%
AA153522	-1.5	0.3	80	159	Serine/threonine protein kinase sulu	Unknown	N
M23501	-1.5	0.2	33	101	TCA3	Unknown	61%
AA063762	-1.5	0.1	112	193	Zinc finger protein 36 homolog (KOX18)	Unknown	63%
AA098588	-1.5	0.1	84	137	Zinc finger protein HRX (ALL-1)	Unknown	57%
W15873	-1.5	0.2	161	258	tctex-1 mRNA	Unknown	61%
AA170748	-1.5	0.1	-14	48	40S Ribosomal protein S4	Unknown	N
W80326	-1.5	0.1	-11	86	Sex-determining protein FEM-1	Unknown	N
AA140159	-1.5	0.2	65	134	Thiol-specific antioxidant protein homolog	Stress response	N
D16492	-1.5	0.1	19	58	RaRF	Unknown	56%
D85845	-1.5	0.2	48	88	Atonal homolog-3	Growth factor	N
L06451	-1.5	0.1	-55	87	Agouti switch protein mRNA	Unknown	100%
AA166500	-1.5	0.2	51	141	Transcriptional regulatory protein RPD3	Unknown	N
L28035	-1.5	0.1	377	578	Protein kinase C-gamma mRNA	Unknown	100%
U52197	-1.4	0.1	296	439	Poly(A) polymerase V	RNA metabolism	N
D29763	-1.4	0.1	799	1130	Seizure-related, product 6 type 3 precursor	Unknown/response	50%
U22015	-1.4	0.1	89	130	Retinoid X receptor interacting protein	Steroid metabolism	100%

*The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

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Table 7. Caloric restriction-related increases in gene expression in neocortex of C57BL/6 mice*

ORF	CR increase	SE	Signal intensity		Gene	Class
			CR	Control		
J04971	4.1	0.7	416	87	Slow/cardiac troponin C (cTnC)	Unknown
D13903	3.1	1.2	150	49	MPTPdelta (type A)	Growth factor
M36660	3.1	0.2	24	-114	NAD(P)H menadione oxidoreductase	Stress response
M55617	3.1	0.6	27	-48	MMCP-4	Unknown
W65178	3.0	0.3	39	-35	BMP-1	Growth factor
AA118682	3.0	0.6	62	-12	Trithorax homolog 2	Transcriptional factor
AA014816	3.0	0.7	257	38	Prolactin homolog	Unknown
U39904	2.9	1.4	100	-169	Citron, putative rho/rac effector	Signal transduction
AA061310	2.9	0.7	87	29	Mitochondrial LON protease	Energy metabolism
U02098	2.8	0.5	82	36	Pur-alpha	DNA metabolism
M29395	2.8	0.3	36	-20	Orotidine-5-monophosphate decarboxylase	DNA metabolism
M23236	2.8	0.5	16	-57	Retrovirus POL protein homolog	Unknown
M13019	2.8	0.4	-15	-130	Thymidylate synthase	DNA metabolism
X76858	2.6	0.4	58	-17	phi AP3	Unknown
W56940	2.5	0.2	81	24	Neuronal-glial cell adhesion molecule homolog	Unknown
X59846	2.4	0.6	215	156	GAS 6	Growth factor
U05247	2.4	0.3	666	250	c-Src kinase	Signal transduction
AA104316	2.3	0.3	25	-46	Type-I ER resident kinase PERK	Stress response
L04302	2.3	0.2	49	2	Thrombospondin 3	Structural
W55507	2.3	0.3	31	-14	D(2) Dopamine receptor	Neurotransmission
AA014909	2.3	0.4	56	-39	Gastrula zinc finger protein XLCGF20.1	Unknown
U46923	2.2	0.8	71	-13	G protein-coupled receptor GPR19	Unknown
M34857	2.2	0.1	176	57	Hox-2.5	Growth factor
M74227	2.2	0.3	162	48	Cyclophilin C (cyp C)	Immune/inflammatory
W12794	2.2	0.3	48	-59	Transforming protein MAF homolog	Transcriptional factor
X62940	2.2	0.1	2199	931	TSC-22	Unknown
L06451	2.2	0.1	136	-55	Agouti switch protein	Unknown
AA052547	2.2	0.1	74	-2	Fatty acid-binding protein, epidermal (E-FABP)	Transport
W17956	2.2	0.4	108	-2	Zinc finger protein 42 homolog	Unknown
X95226	2.2	0.4	53	-1	Dystrobrevin	Structural
AA152808	2.2	0.2	141	24	Proteine kinase PASK	Signal transduction
AA014512	2.1	0.5	32	-3	Unknown	Unknown
W74811	2.1	0.4	17	-46	Apolipoprotein c-II precursor (APO-CII)	Transport
U69270	2.1	0.7	323	210	LIM domain binding protein 1 (Ldb1)	Growth factor
W54720	2.1	0.2	100	19	Ca^{++}-transporting ATPase (brain isoform 1)	Unknown
X13460	2.1	0.1	313	151	Annexin VI	Signal transduction
U61362	2.1	0.3	57	-35	Groucho-related gene 1 protein (Grg1)	Unknown
W09323	2.1	0.3	91	-11	Endothelin-2 precursor (ET-2)	Unknown
W70403	2.1	0.2	17	-19	maiF	Unknown
AA071685	2.0	0.4	93	47	Elongation factor 1-alpha chain homolog	Protein metabolism
W14673	2.0	0.4	133	8	BAT3	Unknown
W53409	2.0	0.3	33	-28	Protein kinase C homolog, alpha type	Signal transduction
U19880	2.0	0.1	28	-6	D4 dopamine receptor gene	Neurotransmission
M75875	2.0	0.4	280	119	MHC H2-K homolog	Unknown
W62842	2.0	0.2	12	-24	ATP synthase lipid-binding protein P2 precursor	Energy metabolism
U48397	2.0	0.3	126	40	Aquaporin 4	Osmotic stress
J00475	2.0	0.3	74	-34	Ig alpha chain region C	Immune/inflammatory
M57960	2.0	0.2	21	-18	Carboxylesterase	Unknown
X57800	2.0	0.1	560	274	PCNA	DNA metabolism
U36277	2.0	0.3	123	70	I-kappa B alpha chain	Stress response

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AA015291	2.0	0.3	140	67	Probable E1-E2 ATPase	Unknown
W82109	2.0	0.3	73	29	Kinesin light chain (KLC)	Transport
M83380	1.9	0.2	25	-25	RelB	Immune/inflammatory
U13174	1.9	0.2	36	2	Basolateral Na-K-2Cl cotransporter	Transport
M33960	1.9	0.2	19	1	Plasminogen activator inhibitor (PAI-1)	Growth factor
X72310	1.9	0.3	106	38	DRTF-polypeptide-1 (DP-1)	Transcriptional factor
AA059886	1.9	0.2	8	-52	Retinal degeneration C protein	Apoptotic factor
U02278	1.9	0.2	19	-32	Hox-B3	Growth factor
AA072842	1.9	0.2	126	72	Na+- and Cl--dependent transporter NTT73	Transport
M98339	1.9	0.2	113	-15	GATA-4	Transcriptional factor
W13427	1.9	0.3	195	94	Platelet factor 4 precursor	Unknown
U44955	1.9	0.2	45	2	Alpha3 connexin gene	Transport
L24191	1.9	0.1	104	25	Intrinsic factor	Transport
W08109	1.9	0.3	142	99	Protein kinase C inhibitor 1 (PKCI-1) homolog	Unknown
W36570	1.9	0.3	146	67	DNA mismatch repair protein MSH2	DNA metabolism
Z34524	1.8	0.2	42	-20	Protein kinase D	Signal transduction
AA105081	1.8	0.2	46	-1	Initiation factor IF-2, mitochondrial	Protein metabolism
U18797	1.8	0.2	95	-3	MHC class I antigen H-2M3	Unknown
M11988	1.8	0.3	141	82	Hox-A6	Growth factor
U17961	1.8	0.2	123	81	p62 ras-GAP associated phosphoprotein	Signal transduction
W85103	1.8	0.1	24	-17	IGF binding protein 4 precursor homolog	Energy metabolism
X07997	1.8	0.2	230	128	MHC class I T-cell antigen Lyt3.1	Immune/inflammatory
W46723	1.8	0.3	164	83	Creatine kinase, B chain homolog	Unknown
W48464	1.8	0.4	18	-7	Protein-tyrosine phosphatase MEG2 homolog	Unknown
L06322	1.8	0.1	84	-4	Delta opioid receptor	Neurotransmission
W49178	1.8	0.1	605	508	Tubulin beta-1 chain homolog	Structural
W48477	1.8	0.2	106	61	Thyrotroph embryonic factor homolog	Unknown
W64225	1.8	0.3	80	44	G21	Unknown
L28167	1.8	0.2	88	45	Zinc finger protein	Unknown
W97199	1.8	0.3	37	62	Negative regulator of transcription subunit 2	Transcriptional factor
X01971	1.8	0.2	20	-35	Interferon alpha 5 (Mu IFN-alpha 5)	Immune/inflammatory
AA061266	1.8	0.3	164	125	Oxysterol-binding protein homolog	Transport
U21855	1.8	0.3	94	31	CAF1	Transcriptional factor
W87078	1.8	0.1	182	90	Unknown	Unknown
W34687	1.8	0.3	188	105	Actin alpha skeletal muscle homolog	Structural
K01238	1.8	0.3	191	127	Interferon alpha 2	Immune/inflammatory
U15635	1.8	0.2	70	9	IFN-gamma induced (Mg11)	Unknown
L13968	1.8	0.1	98	26	UCR-motif DNA-binding protein	Transcriptional factor
M86567	1.8	0.2	122	60	GABA-A receptor alpha-2 subunit	Neurotransmission
M87861	1.8	0.3	51	-22	Granule membrane protein 140	Structural
W55350	1.8	0.3	14	-4	Phosphatidylinositol transfer protein B isoform	Unknown
L43567	1.8	0.1	35	-21	B-cell receptor gene	Immune/inflammatory
AA153196	1.8	0.2	55	-19	Ubiquitin-activating enzyme E1 homolog	Protein metabolism
M28312	1.8	0.1	109	41	Metalloprotease inhibitor TIMP1	Immune/inflammatory

*The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old CR/old control mice. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

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Table 8. Caloric restriction-related decreases in gene expression in neocortex of C57BL/6 mice*

ORF	CR Decrease	SE	Signal Intensity		Gene	Class
			CR	Control		
X76505	-7.2	1.0	-195	73	Tyro 10	Signal transduction
U43088	-6.3	1.1	-109	164	IL-17 (CTLA-8)	Immune/inflammatory
W50186	-5.6	2.1	-38	129	Heavy chain homolog	Unknown
Y07711	-3.5	0.5	28	151	Zyxin	Signal transduction
Z47205	-3.1	0.8	45	200	PLZF	Transcriptional factor
AA000203	-2.8	0.7	-93	26	Corticosteroid-binding globulin precursor	Transport
W83658	-2.6	0.5	51	197	Guanine nucleotide-binding protein G(I)/G(S)/G(O) homolog	Signal transduction
L46815	-2.6	0.2	8	67	Ig kappa chain recombination and transcription enhancer	DNA metabolism
AA153484	-2.4	0.5	208	456	SERCA2	Ion transport
W51466	-2.4	0.4	12	147	Chlorine channel protein P64 homolog	Unknown
U27398	-2.4	0.4	39	132	XPC	DNA Metabolism
X58069	-2.2	0.7	54	164	H2A.X	DNA metabolism
U50712	-2.2	0.4	54	156	MCP-5	Immune /inflammatory
M61909	-2.1	0.3	39	125	NF-kappa-B p65	Stress response
AA072643	-2.1	0.4	49	110	Midkine precursor homolog	Stress response
L01991	-2.1	0.3	48	132	PANG	Unknown
L04678	-2.1	0.2	-64	138	Integrin beta 4 subunit	Structural
W64628	-2.1	0.4	62	197	Guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-7 subunit	Signal transduction
X54098	-2.0	0.3	55	136	lamin B2	Structural
AA023458	-2.0	0.3	20	107	Heat shock 27 KD protein homolog	Stress response
D63380	-2.0	0.2	-19	32	Alpha-1,3-fucosyltransferase	Protein metabolism
U15548	-2.0	0.3	-30	42	Beta 2 thyroid hormone receptor	Energy metabolism
AA123385	-2.0	0.2	57	117	Phosphorylase B kinase gamma catalytic chain	Energy metabolism
X57349	-2.0	0.4	-10	49	Transferrin receptor	Transport
D00659	-2.0	0.1	1	35	Aromatase P450	Biosynthesis
AA028875	-2.0	0.2	-32	54	Glycine-rich cell wall structural homolog	Lysosomal
X76291	-2.0	0.1	11	79	Ihh (Indian Hedgehog)	Signal transduction
AA041982	-1.9	0.3	44	84	LARK	Circadian regulation
AA118758	-1.9	0.2	103	206	Multifunctional aminoacyl-tRNA synthetase	Protein synthesis
W75353	-1.9	0.3	90	162	Apolipoprotein C-IV	Transport
W55410	-1.9	0.2	30	111	Tubulin gamma chain homolog	Unknown
L20343	-1.9	0.2	22	102	L-type calcium channel beta 2a subunit isoform	Transport
W91095	-1.9	0.5	44	93	Valyl-tRNA synthetase	Protein metabolism
X81593	-1.9	0.1	53	119	Winged-helix domain	Transcriptional factor
M38248	-1.9	0.2	-6	25	BALB8N	Unknown
J04694	-1.8	0.3	48	134	Alpha-1 type IV collagen	Structural
L47650	-1.8	0.3	50	85	STAT6 R	Immune /inflammatory
AA023595	-1.8	0.1	38	133	Frizzled protein precursor	Signal transduction
AA015168	-1.8	0.2	42	97	Interferon-gamma receptor beta chain homolog	Immune /inflammatory
AA013951	-1.8	0.1	32	38	Creatine transporter homolog	Energy metabolism
W78443	-1.8	0.2	17	106	MKP-X	Signal transduction
D31842	-1.8	0.2	66	126	PTP36	Structural
W50138	-1.8	0.2	1	162	Putative serine/threonine-protein kinase B0464.5	Unknown
L35307	-1.8	0.2	33	104	c-Krox	Transcriptional factor
AA073154	-1.8	0.3	31	68	Alpha-catenin homolog	Structural
W12720	-1.8	0.3	149	251	RAP-2B homolog	Signal transduction
AA170169	-1.8	0.2	-17	37	Elongation factor 1-gamma homolog	Protein metabolism
W48951	-1.8	0.3	8	30	Voltage-dependent anion-selective channel protein 2 homolog	Unknown
M35732	-1.8	0.3	-13	17	Seminal vesicle secretory protein IV	Unknown

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AA145515	-1.8	0.3	68	187	Pre-MRNA splicing factor PRP6	RNA metabolism
W13162	-1.8	0.1	-7	62	Cell division protein kinase 4	DNA metabolism
J03482	-1.8	0.2	42	113	Histone H1	DNA metabolism
W82793	-1.8	0.1	-4	59	Topoisomerase E III homolog	DNA metabolism
Z31360	-1.8	0.3	1	51	P/L01	Unknown
Y09632	-1.8	0.1	16	37	Rabkinesin-6	Transport
AA066621	-1.8	0.2	13	63	60S ribosomal protein L10	Protein metabolism
U67874	-1.8	0.3	46	85	Ubiquitin thioesterase family	Protein metabolism
AA109714	-1.8	0.3	562	968	SKP1	RNA metabolism
AA007957	-1.8	0.2	210	357	Threonyl-tRNA synthetase homolog	Protein metabolism
AA162633	-1.8	0.2	46	95	Isoleucyl-tRNA synthetase	Protein metabolism
M17299	-1.8	0.3	29	101	Phosphoglycerate kinase (pgk-2)	Energy metabolism
AA050102	-1.7	0.3	211	263	Elongation factor 2 (EF-2)	Protein metabolism
W54637	-1.7	0.2	72	137	Tubulin beta-2 chain class-II homolog	Unknown
D10028	-1.7	0.3	167	312	Glutamate receptor channel subunit zeta 1	Neurotransmission
M28587	-1.7	0.2	-52	30	Alpha leukocyte interferon	Immune /inflammatory
AA023506	-1.7	0.2	60	144	Insulin receptor substrate-3	Energy metabolism
W70629	-1.7	0.3	92	158	COPII	Protein metabolism
U33626	-1.7	0.3	66	125	PML isoform 1 (Pml)	Unknown
AA144746	-1.7	0.2	42	92	EF-1-delta	Protein metabolism
M19380	-1.7	0.3	1406	2303	Calmodulin (Cam III)	Signal transduction
AA144136	-1.7	0.2	43	100	Choline kinase R1 homolog	Biosynthesis
AA165847	-1.7	0.3	331	509	EF-1-alpha2 homolog	Protein metabolism
W33415	-1.7	0.2	90	136	ATP citrate-lyase	Unknown
U35233	-1.6	0.1	71	109	Endothelin-1	Vasoconstrictive peptide
W57384	-1.9	0.3	6	15	ATP synthase A chain homolog	Energy metabolism
X60452	-1.6	0.3	124	200	Cytochrome P-450IIIA	Stress response
AA022127	-1.6	0.1	172	279	Vascular endothelial growth factor	Unknown
AA168841	-1.6	0.2	169	289	Serine/threonine-protein kinase PAK	Unknown
AA120586	-1.6	0.1	9	64	Apolipoprotein B-100 precursor	Stress response
AA104561	-1.6	0.2	104	166	EIF-4A homolog	Protein metabolism
X17071	-1.6	0.1	25	90	Trophoblast-specific protein	Growth factor
M96265	-1.6	0.1	153	250	Galactose-1-phosphate uridyl transferase	Biosynthesis
AA145160	-1.6	0.2	178	287	Translational initiation factor 2 alpha	Protein metabolism
X63473	-1.6	0.1	69	110	m4 muscarinic acetylcholine receptor	Neurotransmission
AA002750	-1.5	0.2	176	290	5-lipoxygenase activating protein (FLAP)	Immune /inflammatory
W64698	-1.5	0.2	51	83	Protein kinase C inhibitor 1	Signal transduction
U63841	-1.5	0.1	120	197	NeuroD3	Growth factors
U04294	-1.5	0.1	99	150	Potassium channel subunit (m-eag)	Transport
M33227	-1.5	0.2	259	396	Cryptdin-related (CRS4C)	Immune /inflammatory
U20532	-1.5	0.1	45	67	P45 NF-E2 related factor 2 (Nrf2)	Transcriptional factor
AA140026	-1.5	0.1	378	519	DNA directed RNA polymerase polypeptide G	DNA metabolism
W09025	-1.5	0.1	47	68	ATP synthase B chain homolog	Energy metabolism
W29163	-1.5	0.1	342	465	Leydig cell tumor 10kd protein homolog	Unknown
AA155191	-1.5	0.1	36	65	Kinesin heavy chain	Transport
M80360	-1.5	0.1	63	96	Rep-3	DNA metabolism
AA044561	-1.4	0.2	93	132	PEP carboxykinase - mitochondrial	Energy metabolism
AA096843	-1.4	0.2	130	175	Unknown	Unknown
X57277	-1.4	0.1	908	1298	Rac1	Signal transduction
W82998	-1.4	0.1	256	363	BUB3	DNA metabolism

*The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old CR/old control mice. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

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Table 9. Aging-related increases in gene expression in the cerebellum of C57BL/6 mice*

ORF	Fold Change	SE	Signal Intensity Old	Young	Gene	Class	CR Prevention
AA120109	9.3	3.4	254	29	Interferon-induced protein 6-16 precursor	Immune/inflammatory	N
M21050	6.4	0.9	291	14	Lysozyme P (Lzp-s)	Immune	88
X56824	5.7	1.9	160	89	Tumor-induced 32 kD protein (p32)	Unknown	100
V00727	5.6	2.6	282	57	c-fos	Stress	30
M13019	4.9	0.7	109	3	Thymidylate synthase	DNA metabolism	87
L16894	4.7	1.0	192	5	Cyclophilin C (CyCAP)	Immune/inflammatory	N
AA146437	4.7	0.3	841	169	Cathepsin S precursor	Stress	62
X58861	4.4	0.2	719	160	C1Q alpha-chain	Immune/inflammatory	80
W67046	4.3	0.8	50	1	C6 chemokine	Immune/inflammatory	N
X66295	4.1	0.6	508	147	C1q C-chain	Immune/inflammatory	56
W65899	4.1	1.8	152	58	Guanine nucleotide-binding protein	Signal transduction	80
U00677	4.1	2.2	16	-10	Syntrophin-1	Neurotransmission	100
X68273	3.9	1.8	108	-37	Macrostalin	Immune/inflammatory	N
U19854	3.9	0.5	35	-63	Ubiquitinating enzyme E2-20K	Protein metabolism	100
U63133	3.9	1.1	318	95	Emv-3	Viral	N
L20315	3.8	0.1	97	26	MPS1	Immune/inflammatory	56
K01347	3.8	0.7	337	109	Glial fibrillary acidic protein (GFAP)	Stress	61
M17440	3.7	0.3	445	116	Sex-limited protein (SlpA)	Immune/inflammatory	N
X91144	3.6	1.3	38	-2	P-selectin glycoprotein ligand 1	Immune/inflammatory	100
U43084	3.5	0.8	54	18	IFIT-2 Glucocorticoid-attenuated response	Immune/inflammatory	N
AA089333	3.4	0.2	208	61	Cathepsin S precursor	Stress	71
X83733	3.4	0.3	71	-7	SAP62-AMH	RNA metabolism	100
W45750	3.3	1.3	197	257	Guanine nucleotide-binding protein G(T)	Signal transduction	100
M22531	3.3	0.2	431	146	Clq B-chain	Immune/inflammatory	65
AA031244	3.1	0.4	83	9	DNAJ protein homolog HSJ1	Stress	100
M60429	3.1	0.8	121	37	Ig-gamma 1 chain	Immune/inflammatory	100
AA036067	3.0	0.4	815	311	Apolipoprotein E precursor (APO-E)	Lipid transport	28
U06119	2.9	0.3	27	4	Cathepsin H prepropeptide (ctsH)	Stress response	55
AA106347	2.9	0.3	243	57	Angiotensinogen precursor	Osmoregulation	80
W98998	2.9	0.7	182	79	Neurogenic locus notch homolog protein 1	Immune/inflammatory	100
AA059700	2.8	0.3	2013	687	MHC class I B(2)-microglobulin	Immune/inflammatory	45
U73037	2.8	0.8	69	41	Interferon regulatory factor 7 (mirf7)	Immune/inflammatory	50
Y00964	2.8	0.3	780	316	beta-hexosaminidase (Hexb)	Unknown	47
X55315	2.8	0.6	63	15	Fetus cerebral cortex for 3UTR	Transcription factor	100
U37465	2.8	0.1	15	-7	Protein tyrosine phosphatase phi (PTPphi)	Unknown	63
L07803	2.7	1.2	24	-15	trombospondin 2	Structural	N
U19119	2.7	0.3	52	-5	G-protein-like LRG-47	Immune/inflammatory	N
X52886	2.6	0.2	893	326	Cathepsin D	Stress response	38
W70578	2.6	1.2	31	7	Antigen WC1.1	Immune/inflammatory	81
X16705	2.6	0.4	93	-4	Laminin B1	Structural	84
W57539	2.6	0.3	28	6	Oocyte zinc finger protein XLCOF8	Unknown	N
X52308	2.6	0.4	32	9	Thrombin	Fibrinogen activation	91
U70859	2.6	0.7	109	46	Cationic amino acid transporter (CAT3)	AA transport	49
U41497	2.6	1.1	160	40	Very-long chain acyl-CoA dehydrogenase	Lipid metabolism	100
AA089339	2.6	0.5	76	31	Cystatin C precursor	Immune/inflammatory	100
X16151	2.5	0.1	239	95	Early T-lymphocyte activation 1 protein	Immune/inflammatory	49
U37419	2.5	0.5	111	-2	G protein alpha subunit (GNA-15)	Unknown	N
K02785	2.5	0.5	15	-6	r-fos	Stress response	N
M12289	2.5	0.5	39	25	Perinatal skeletal myosin heavy chain	Structural	100
X58849	2.4	0.4	59	13	Murine Hox-4.7	Developmental	100
AA063858	2.4	0.2	89	32	Rho-related GTP-binding protein RHOG	Signal transduction	74
D10632	2.4	0.2	33	-27	Zinc finger protein	Transcription factor	N
U33005	2.3	0.4	35	-8	tbc1	Unknown	N
W85160	2.3	0.7	70	41	40S ribosomal protein S4, X isoform	Unknown	100
U57331	2.3	1.0	42	15	Transcription factor Tbx6 (tbx6)	Developmental	92

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U44731	2.3	0.2	71	20	Putative purine nucleotide binding protein	...une/inflammatory	N
W87253	2.3	0.6	58	16	Integrin beta-5 subunit precursor	Cell adhesion	100
U53142	2.3	0.2	223	101	Endothelial constitutive nitric oxide synthase	Neurotransmission	N
AA087715	2.3	0.1	85	-61	GTPase-activating protein SPA-1	Unknown	N
D49429	2.3	0.3	554	251	Rad21 homolog	DNA metabolism	73
AA155318	2.3	0.4	291	129	HNRP1	RNA metabolism	N
AA032593	2.3	0.1	99	17	Transducin beta chain 2	Signal transduction	83
X03690	2.3	0.2	45	-13	Ig mu chain	Immune/inflammatory	93
M26417	2.3	0.5	54	28	T cell receptor beta chain	Immune/inflammatory	100
X86374	2.2	0.6	73	38	TAG7	Immune/inflammatory	38
W90894	2.2	0.3	27	-11	Cell division protein kinase 4	DNA metabolism	100
M84005	2.2	0.7	83	51	Olfactory receptor 15	Odor receptor	23
X55573	2.2	0.5	55	19	Brain-derived neurotrophic factor	Growth factor	N
W30129	2.2	0.3	90	-16	Phosphatidylinositol glycan hmolog	Structural	100
AA163771	2.2	0.3	153	67	EIF-2B epsilon subunit	Protein metabolism	N
X72910	2.1	0.4	96	44	HSA-C	Unknown	N
AA116604	2.1	0.2	303	181	Cathepsin Z	Stress response	64
L16462	2.1	0.4	51	4	BCL2-related protein A1	Apoptosis	58
L13732	2.1	0.4	53	29	Nall. resistance-asstd. macrophage protein1	Immune/inflammatory	85
D37791	2.1	0.1	934	424	Beta-1,4-galactosyltransferase	Protein metabolism	82
AA125097	2.0	0.1	618	313	Unknown	Unknown	94
AA109998	2.0	0.2	40	12	Hexokinase D homolog	Energy metabolism	100
M88127	2.0	0.2	33	-8	APC2 homolog	Unknown	82
X13538	2.0	0.5	114	45	Hox-1.4	Growth/development	100
V01527	2.0	0.5	28	10	H2-IA-beta	Immune/inflammatory	100
AA144411	2.0	0.1	86	79	Unknown	Unknown	100
X63535	2.0	0.1	55	21	Tyrosine-protein kinase receptor UFO	Signal transduction	N
M83348	2.0	0.1	42	22	Pregnancy specific glycoprotein homolog	Unknown	N
W08211	2.0	0.2	62	26	TGF-beta receptor type III	Signal transduction	100
W13136	2.0	0.4	266	87	Angiotensinogen	Osmoregulation	36
W46084	2.0	0.1	89	45	Unknown	Unknown	N
U73744	2.0	0.1	3958	2909	Heat shock 70	Stress response	100
D29763	1.9	0.2	465	271	Seizure-related, product 6 type 3	Unknown	47
AA118121	1.9	1.0	51	37	Isoleucyl-tRNA synthetase	Protein metabolism	N
M27034	1.9	0.2	258	163	MHC class 1 D-region	Immune/inflammatory	N
U35249	1.9	0.1	68	36	CDK-activating kinase assembly factor	DNA metabolism	61
J03776	1.9	0.4	37	22	Down regulatory protein (rpl-1r) of IL-2 receptor	Immune/inflammatory	N
U28728	1.9	0.3	221	112	Ets	Signal transduction	66
AA124192	1.9	0.2	411	244	Unknown	Unknown	44
W63809	1.8	0.4	136	80	Unknown	Unknown	73
X16834	1.8	0.2	455	182	Galectin-3	Immune/inflammatory	N
X16995	1.8	0.2	351	221	N10 nuclear hormonal receptor homolog	Unknown	100
J02870	1.8	0.2	848	380	40S ribosomal protein SA	Protein metabolism	100
L21768	1.8	0.2	153	76	EGF15	Growth factor	68
AA117284	1.8	0.1	217	123	Zinc finger protein homolog	Unknown	N

*The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calcuted for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

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Table 10. Aging-related decreases in gene expression in the cerebellum of C57BL/6 mice*

ORF	Fold Change	SE	Signal Old	Intensity Young	Gene	Class	CR Prevention
U00445	-4.3	1.4	39	132	Glucose-6-phosphatase	Energy metabolism	79
W48504	-4.1	1.1	32	78	phosphoneuroprotein 14 homolog)	Unknown	N
AA153337	-3.9	0.7	67	218	Myosin regulatory light chain 2 (MLC-2).	Unknown	61
W51213	-3.9	0.5	14	57	NEDD-4 homolog	Protein metabolism	55
X56304	-3.1	0.4	2	27	Tenascin	Growth/development	N
W12681	-3.1	0.6	30	126	Hepatocyte growth factor	Growth/development	37
Z68889	-2.9	1.0	30	70	Wnt-2 homolog	Growth/development	N
W55684	-2.8	0.6	13	37	Brain protein I47	Unknown	N
U04827	-2.8	0.5	94	219	Brain fatty acid-binding protein (B-FABP)	Growth/development	N
AA008066	-2.7	1.0	1	61	Pre-mRNA splicing factor PRP22	Unknown	74
W55300	-2.7	0.7	20	47	Fatty acid-binding protein, heart (H-FABP)	Unknown	71
D13903	-2.7	0.5	7	37	MPTPdelta (type A)	Growth/development	N
AA013976	-2.6	0.5	162	405	POL polyprotein; reverse transcriptase; ribonuclease H	Unknown	N
W10865	-2.6	0.2	14	142	Myosin light chain 1, atrial/foetal isoform	Unknown	N
AA020296	-2.5	0.2	-162	166	NG9	Growth/development	100
W64865	-2.5	1.1	10	31	Stat-3	Unknown	N
AA139694	-2.5	0.3	64	203	Beta-myosin heavy chain	Transport	100
U29762	-2.5	0.3	304	657	Albumin gene D-Box binding protein	Transcription Factor	N
M87276	-2.4	0.5	16	34	Thrombospondin	Structural	52
X02677	-2.4	0.2	63	160	Anion exchange protein	Anion exchanger	100
X04836	-2.4	0.2	22	68	T-cell antigen CD4	Immune/inflammatory	100
X87242	-2.4	0.3	48	111	unc-33	Growth/development	70
AA163021	-2.4	0.2	28	143	Annexin VIII	Signal transduction	84
M31810	-2.4	0.3	29	113	P-protein membrane transporter	Transport	100
M97900	-2.4	0.6	18	49	Unknown	Unknown	20
M15008	-2.4	0.6	101	227	Steroid 21-hydroxylase B	Steroid metabolism	100
M99377	-2.4	0.5	77	191	Alpha-2 adrenergic receptor	Neurotransmission	N
M32490	-2.4	0.3	62	122	Cyr61	Growth/development	41
AA168350	-2.3	0.3	130	237	Cysteinyl-tRNA synthetase	Protein metabolism	83
AA061206	-2.3	0.2	8	52	Unp (ubiquitin protease)	Protein metabolism	N
W12794	-2.3	0.3	23	96	Unknown	Unknown	78
AA050593	-2.3	0.1	5	69	Unknown	Unknown	62
AA050715	-2.3	0.3	64	148	Smoothelin	Structural	92
AA106463	-2.2	0.3	110	277	Phosphoenolpyruvate carboxykinase.	Energy metabolism	N
X90829	-2.2	0.3	-16	9	Lbx1	Growth/development	N
X65588	-2.2	0.3	-1	24	mp41	Neurotransmission	N
J00475	-2.2	0.2	-23	58	Ig alpha chain	Immune/inflammatory	N
X03019	-2.2	0.3	4	71	GM-CSF	Immune/inflammatory	26
W34687	-2.2	0.4	62	115	Alpha-actin	Transport	78
W75614	-2.2	0.4	27	56	Alpha-synuclein	Growth/development	N
AA068153	-2.2	0.3	14	39	Polyadenylate-binding protein	RNA metabolism	55
U36842	-2.1	0.5	22	36	Riap 3-inhibitor of apoptosis	Apoptosis	100
W09127	-2.1	0.3	3	85	60S ribosomal protein L22	Protein metabolism	100
D63819	-2.1	0.2	29	87	Neuropeptide Y-Y1 receptor	Neurotransmission	N
M33884	-2.1	0.1	70	139	Env polyprotein	Viral protein	55
AA144430	-2.1	0.3	64	156	NF-KB P100 inhibitory subunit	Stress response	48
AA168554	-2.1	0.3	119	246	Unknown	Unknown	85
U35730	-2.1	0.8	12	30	Jerky	Unknown	N
M92649	-2.1	0.4	45	112	nitric oxide synthase	Neurotransmission	N
D12907	-2.1	0.2	55	126	Serine protease inhibitor homologue	Unknown	85
M17327	-2.1	0.2	234	566	Env polyprotein	Viral protein	56
AA170444	-2.1	0.2	172	246	Ubiquitin-activating enzyme E1	Protein metabolism	100
W12658	-2.1	0.3	203	415	FKBP-rapamycin associated protein	Unknown	N
AA123026	-2.1	0.3	60	116	REG 2	Unknown	100

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W13125	-2.1	0.5	111	232	Phenylalanyl-tRNA synthetase beta chain	Protein metabolism	N
AA103862	-2.1	0.4	53	143	Unknown	Unknown	N
U21301	-2.1	0.6	30	62	c-mer tyrosine kinase receptor	Signal transduction	N
W13586	-2.1	0.1	29	136	Myosin light chain 1 homolog	Transport	100
W42217	-2.1	0.1	69	143	Ribosomal protein S20	Protein metabolism	100
AA153522	-2.1	0.4	95	191	Serine/threonine kinase	Signal transduction	78
W30612	-2.0	0.1	70	160	Chloride intracellular channel 3	Transport	100
W11621	-2.0	0.4	78	138	Zinc finger protein 126	Unknown	N
X72805	-2.0	0.3	25	63	CD-1 histone H1t	DNA metabolism	N
L08407	-2.0	0.3	38	117	Collagen type XVII	Structural	N
AA145609	-2.0	0.2	55	134	cAMP responsive element modifier	Transcriptional factor	34
W12756	-2.0	0.1	48	117	Unknown	Unknown	92
W75523	-2.0	0.3	48	95	Vertebrate homolog of C. elegans Lin-7 type 2	Unknown	N
D85904	-1.9	0.3	69	129	Heat shock 70-related protein Apg-2	Stress response	N
AA138911	-1.8	0.2	176	311	RNA helicase PRP16	RNA metabolism	100
W42216	-1.8	0.1	183	361	SWI/SNF related homolog	Transcriptional factor	74
W12395	-1.8	0.4	141	237	Transcription elongation factor A (SII)	Transcriptional factor	88
K03235	-1.8	0.1	84	149	Prolifenn 2	Growth factor	100
AA145859	-1.8	0.1	4110	5250	Unknown	Unknown	100
W57194	-1.8	0.2	61	108	Ubiquitin carboxyl terminal hydrolase 12	Protein metabolism	N
AA166440	-1.7	0.1	229	389	Phosphatidylserine decarboxylase	Protein metabolism	N
L33726	-1.7	0.1	69	128	Fascin homolog 1	Structural	100
L35549	-1.7	0.4	30	38	Y-box binding protein homolog	Unknown	100
AA154514	-1.7	0.1	7639	12878	ATP synthase A chain (protein 6) homolog	Energy metabolism	100
AA143937	-1.7	0.1	384	697	Beta-centractin	Transport	70
AA027387	-1.7	0.1	169	270	Rab-4B	Transport	51
L38971	-1.7	0.2	205	334	Integral membrane protein 2	Unknown	43
W10526	-1.7	0.1	193	301	Ca^{++} channel, voltage-dep., gamma subunit 1	Transport	90
W12204	-1.6	0.2	114	200	Ca2+/calmodulin-dependent protein kinase isoform gamma B	Signal transduction	N
AA170173	-1.6	0.1	149	289	NTT-73	Transport	100
M64403	-1.6	0.1	126	208	Cyclin D1 homolog	DNA metabolism	100
W13191	-1.6	0.1	288	347	Thyroid hormone receptor alpha 2	Energy metabolism	87
U47543	-1.6	0.1	121	205	NGF1-A binding protein 2 (NAB2)	Growth factor	N
D70848	-1.6	0.2	154	246	Zic2 (cerebellar zinc finger protein)	Neural development	77
X56518	-1.6	0.3	106	164	Acetylcholinesterase	Neurotransmission	N
AA144588	-1.6	0.2	233	368	Beta-adrenergic receptor kinase 2 homolog	Neurotransmission	33
AA139828	-1.6	0.1	224	351	gonadotropin inducible transcription repressor-1 homolog	Unknown	100
AA061170	-1.6	0.2	43	65	WW-domain oxidoreductase homolog	Unknown	N
X58287	-1.6	0.3	84	153	mR-PTPu	Signal transduction	N
L13129	-1.6	0.1	162	220	Annexin A7	Exocytosis	90
D85037	-1.6	0.1	50	77	Doc2beta	Neruotransmission	N
U30823	-1.6	0.2	55	102	Myocyte enhancer factor-2A	Transcriptional factor	33
W64791	-1.6	0.1	92	143	Galactokinase	Energy metabolism	N
X52622	-1.6	0.1	274	377	IN	Viral protein	100
AA063914	-1.5	0.1	175	267	Alpha-tubulin	Transport	64

*The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. . The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

BNSDOCID: <WO_____0112851A2_I_>

Table 11. Genes upregulated by aging in C57BL/6 mice heart from Mu19K GeneChip

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC27774	396	218	490	-1328	-2197	-1280	25.8
TC35932	71	1391	355	-596	-507	-1500	17.2
TC39719	938	595	1380	529	-129	-562	14.6
TC24697	1510	2431	3697	173	-823	-537	13.9
TC17809	4141	4286	4415	224	369	921	11.0
TC28794	1358	1313	1445	349	-38	657	10.4
TC16257	439	867	471	-121	-528	166	10.3
TC34515	1687	1117	966	465	-1068	-1737	9.4
TC29214	102	154	188	-381	-122	-209	9.0
TC32857	733	915	524	200	82	90	8.3
TC37114	553	803	466	377	-99	59	8.2
TC17940	947	1889	1474	-54	160	-1487	8.1
TC39890	912	1658	1190	639	617	8	7.7
TC39498	1080	738	1754	-29	634	-462	7.3
TC25820	340	510	325	-353	-315	-575	6.1
TC24908	12482	8941	7330	1337	1838	1387	5.8
TC29305	1271	1020	827	841	382	606	5.5
TC16024	739	1570	995	603	312	123	4.8
TC33899	304	287	240	64	30	73	4.8
TC16184	1294	3064	3523	428	388	447	4.7
TC39399	338	421	286	-81	208	27	4.5
TC17839	1506	946	2315	248	512	146	4.5
TC18386	1822	1967	1585	281	566	477	4.4
TC27769	3796	5647	3986	1260	975	2286	4.4
TC37583	433	617	758	119	425	93	4.3
TC22269	6795	7593	8793	920	2322	5205	4.1
TC28239	2039	1359	881	227	495	604	4.1
TC34440	340	310	258	21	-437	-170	4.1
TC39301	803	1692	1539	27	710	778	4.1
TC29662	997	2372	1701	174	650	694	4.0
TC33757	339	323	257	49	76	231	3.9
TC29977	858	631	879	102	541	335	3.9
TC19997	419	358	384	84	67	266	3.8
TC27675	4002	5625	6693	1292	1580	1426	3.8
TC21921	677	779	864	339	43	229	3.8
TC41800	915	441	1157	-8	69	180	3.7
TC31694	2158	2467	2245	449	306	976	3.7
TC28855	282	194	355	67	127	62	3.6
TC31277	311	243	445	44	182	172	3.6
TC21628	176	422	304	124	76	68	3.5
TC36063	498	623	390	-80	346	-52	3.5
TC33608	514	449	479	140	165	124	3.4
TC38147	420	212	473	61	173	211	3.3
TC23622	112	328	186	-55	60	99	3.2
TC34697	549	450	752	89	356	370	3.2
TC22213	1892	2305	2099	655	730	644	3.1
TC31569	282	113	247	73	127	4	3.1
TC28942	517	1055	1020	301	364	224	3.0

3NSDOCID: <WO_____0112851A2_I_>

Table 12. Genes downregulated by aging in C57BL/6 mice heart from Mu19K GeneChip

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC27282	20	-2020	-2141	5078	970	879	-86.2
TC32064	-217	-844	-511	2335	2211	2176	-58.6
TC24160	-1155	-3091	-2382	427	4103	4674	-56.2
TC14603	867	-2795	-2128	4729	2680	2255	-53.4
TC22507	-1155	-1599	-1409	1319	2177	2942	-50.4
TC15929	-1203	-1586	-1787	1348	1014	2026	-47.0
TC19943	-687	-669	-428	2880	2552	1067	-41.7
TC18736	-1142	787	-1647	2711	3654	4006	-33.0
TC19957	1242	-501	958	6796	6771	5343	-30.5
TC37452	175	-1172	-441	820	2013	1233	-27.3
TC33452	532	-740	-465	2021	880	719	-26.3
TC14870	-289	-1650	-2496	30	209	1249	-25.2
TC26312	-118	-73	-146	406	1251	1344	-24.3
TC25802	-688	-736	-1968	31	707	695	-23.7
TC14624	-227	-943	-758	1675	718	352	-22.6
TC41568	-684	-3089	-1954	7	711	129	-22.6
TC16488	-1548	-57	-1609	1055	1739	190	-22.5
TC18539	122	1114	-269	3415	2604	2614	-21.6
TC37617	-1738	-296	-2150	2156	2231	422	-20.6
TC39618	-56	-204	-168	769	1196	887	-19.5
TC37350	-1070	-657	-655	1944	1258	260	-19.5
TC36639	1496	-3251	-23	4489	2756	6211	-19.4
TC16420	48	-674	-17	1059	1053	1072	-18.6
TC37529	177	151	333	6190	3159	2499	-18.3
TC15736	-67	-1109	-1133	242	530	647	-18.2
TC36992	498	-2096	-450	2140	2451	1214	-17.9
TC28761	326	-105	847	4047	2990	1712	-17.9
TC25360	-1421	-2210	-2177	332	173	204	-17.2
TC16633	-66	-612	-638	626	240	496	-17.0
TC18250	145	-416	-464	2429	890	804	-16.3
TC35586	-337	-526	6	762	782	328	-16.2
TC37067	2006	137	2589	7334	6130	5348	-16.0
TC40509	176	-216	197	2219	724	1177	-15.9
TC37745	380	-1137	141	822	1566	1043	-15.8
TC24220	648	227	48	1916	1805	2138	-14.9
TC17700	159	-80	-657	565	810	690	-14.4
TC17256	-2800	-3715	-3550	629	2754	950	-13.4
TC37672	-117	427	247	1149	1712	1737	-13.0
TC18637	202	-208	-312	1012	907	794	-12.8
TC15863	-639	250	289	882	794	1198	-12.7
TC23647	-575	334	-1428	1821	2149	2101	-12.5
TC16841	375	-198	430	1177	1044	1257	-12.3
TC27576	-70	75	428	596	1326	857	-12.2
TC21963	-281	-437	-368	944	136	231	-12.2
TC36608	-527	-316	-140	343	254	7	-12.1
TC26887	60	188	-100	589	933	734	-11.9
TC24501	539	518	79	4279	1947	1811	-11.8
TC36239	902	-102	843	1587	1899	2152	-11.3
TC38050	-47	-81	115	324	633	645	-11.3
TC37660	-1	-617	-203	450	240	314	-11.1
TC34986	-1	-98	-28	726	315	235	-10.7
TC30885	402	-55	27	878	734	398	-10.4
TC16723	478	276	62	1703	1736	1138	-10.3
TC20671	-70	-827	-303	948	1087	410	-10.2
TC14753	-332	-265	-325	418	335	276	-10.1

BNSDOCID: <WO_____0112851A2_I_>

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC16229	-156	515	107	1224	681	1077	-10.1
TC24641	-372	-382	-329	127	845	718	-10.0
TC35052	139	-86	-19	504	459	447	-9.9
TC20554	158	392	625	1255	896	1199	-9.8
TC25572	-470	-460	-871	472	1340	791	-9.5
TC21262	220	-336	1193	2061	1581	2928	-9.5
TC25416	48	-285	-104	487	554	460	-9.5
TC41297	373	-176	455	1093	976	991	-9.4
TC37701	-219	-338	-398	830	294	236	-9.4
TC34944	364	462	369	3507	3271	3393	-9.3
TC31449	-7	53	-51	300	252	217	-9.0
TC41997	167	-142	199	682	1057	893	-8.8
TC36033	-164	-295	-678	1048	194	241	-8.8
TC27468	584	492	560	1011	1031	929	-8.8
TC16039	603	-2181	-1612	2105	1544	1004	-8.6
TC19352	-918	-290	-600	1103	700	859	-8.5
TC25041	229	-697	-295	726	515	558	-8.4
TC35104	548	1	563	1294	1692	715	-8.3
TC25357	143	-277	-40	897	788	1407	-8.0
TC22194	119	-63	-176	477	440	633	-7.9
TC20469	284	-303	-850	1031	591	674	-7.7
TC41078	-35	-289	42	551	232	148	-7.7
TC39603	417	-253	300	813	952	586	-7.6
TC36846	64	-83	117	606	487	353	-7.2
TC24619	-11	-273	-224	212	483	418	-7.1
TC15831	1167	1269	87	3253	1942	1814	-7.1
TC25629	-4	-309	-341	387	106	167	-7.1
TC23144	-91	-175	-322	770	114	393	-7.0
TC29553	77	-27	-110	93	283	185	-7.0
TC36286	-312	-574	-44	702	929	668	-6.8
TC23964	1265	1225	276	6611	4409	5007	-6.8
TC37675	19	103	139	408	734	469	-6.6
TC41144	236	58	273	1095	734	708	-6.6
TC40883	-31	-251	88	201	473	370	-6.6
TC27606	-640	-765	-579	232	208	394	-6.5
TC14712	1140	643	-15	1661	1331	2644	-6.5
TC26859	803	95	985	3249	2325	2184	-6.4
TC33246	168	-216	-384	517	283	384	-6.4
TC37343	180	-27	34	459	508	346	-6.3
TC37275	1193	720	808	1722	1828	1992	-6.3
TC18134	685	695	488	145	57	96	-6.2
TC40210	166	-245	91	354	502	400	-6.1
TC17241	438	-110	756	1750	2691	2519	-6.1
TC21038	133	-138	-206	600	218	168	-6.1
TC22355	12	-396	-116	182	232	177	-6.1
TC38075	111	-40	11	533	588	613	-6.0
TC38184	-263	-107	58	293	235	92	-6.0
TC37491	239	166	349	1404	1500	1141	-5.9
TC33420	-132	-208	-114	388	128	88	-5.9
TC37318	1331	188	833	1241	3321	2861	-5.8
TC37916	-273	-62	-202	198	55	43	-5.8
TC17885	-178	169	-288	1591	1472	1445	-5.7
TC15884	390	-134	-109	734	431	493	-5.6
TC40452	-94	-141	107	291	339	359	-5.6
TC29330	512	370	140	2164	1174	930	-5.6
TC17616	101	46	57	531	853	808	-5.6

INSDOCID: <WO_____0112851A2_I_>

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC21414	-62	-2	-143	111	296	344	-5.5
TC17717	36	-83	-144	222	172	209	-5.4
TC31495	156	155	77	280	502	371	-5.3
TC18144	2048	819	1400	3236	3117	3190	-5.3
TC19650	-120	-282	-56	358	86	18	-5.2
TC25815	36	224	90	490	506	508	-5.2
TC37544	470	242	458	527	767	691	-5.1
TC38870	119	-35	187	1057	704	587	-5.1
TC26789	111	49	-68	240	243	270	-5.0
TC37493	103	250	396	993	982	795	-5.0
TC41579	465	120	253	959	557	669	-5.0
TC17620	326	452	303	721	565	788	-4.9
TC18572	29	-130	-51	208	264	348	-4.9
TC41021	217	84	43	611	329	306	-4.9
TC25021	61	95	69	471	440	235	-4.9
TC37829	-235	-243	92	142	292	771	-4.7
TC19783	35	-10	249	371	604	767	-4.6
TC24373	-111	-424	171	376	384	395	-4.6
TC41191	54	-407	-30	741	36	721	-4.6
TC30942	281	146	19	1772	1068	1025	-4.5
TC14554	28	-147	44	651	479	471	-4.5
TC32618	210	68	260	435	504	448	-4.5
TC35574	1063	295	1619	2598	3642	3046	-4.5
TC39584	1090	1014	538	2430	3908	4185	-4.4
TC37290	-26	-15	90	541	212	211	-4.3
TC14567	968	216	267	2605	1842	1044	-4.2
TC30986	66	-14	76	306	151	178	-4.2
TC35356	211	-3	224	474	598	338	-4.2
TC35554	91	-100	89	572	566	558	-4.2
TC22851	810	416	520	3098	1773	1661	-4.2
TC20860	316	118	498	1291	739	695	-4.1
TC41573	212	88	343	656	1162	931	-4.1
TC32333	471	489	542	2274	1696	1350	-4.1
TC20845	164	222	-12	508	438	361	-4.0
TC37484	192	-14	236	408	384	494	-4.0
TC33993	-342	-140	-253	161	567	752	-4.0
TC37769	670	107	485	2676	1219	1617	-3.9
TC31667	435	73	167	1141	556	585	-3.9
TC18679	1123	1055	1090	638	626	366	-3.9
TC21666	5	81	-153	203	351	195	-3.8
TC41350	213	83	206	680	403	479	-3.8
TC21304	-109	-65	-63	243	38	61	-3.7
TC39507	-137	-208	-77	310	61	22	-3.7
TC19129	827	722	469	1364	1364	1142	-3.6
TC21197	-376	-1186	-1054	1746	1222	416	-3.6
TC38888	67	8	50	292	106	199	-3.6
TC32452	992	974	1165	2411	2887	2965	-3.5
TC14511	739	660	298	942	1924	2211	-3.5
TC29246	716	546	538	1125	991	1222	-3.4
TC15902	137	-4	55	350	211	209	-3.4
TC37774	378	234	424	1148	1146	952	-3.3
TC27288	377	394	816	1451	1663	1554	-3.3
TC31668	-76	-153	-46	170	103	10	-3.3
TC41983	252	-1	190	240	490	429	-3.3
TC14823	933	420	557	1168	2494	1983	-3.3
TC40714	416	939	354	1914	1744	1041	-3.3

BNSDOCID: <WO_____0112851A2_I_>

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC20259	272	22	86	330	285	513	-3.3
TC23344	462	577	862	1602	2043	2131	-3.3
TC27282	1068	765	508	3300	1911	1689	-3.2
TC21501	500	1332	782	4505	3307	3468	-3.2
TC34693	-14	177	761	1242	1088	1137	-3.2
TC41186	231	120	272	1122	579	641	-3.1
TC26140	276	-43	141	279	541	452	-3.1
TC20981	-59	-53	-38	137	67	86	-3.1
TC39851	97	-176	80	457	204	169	-3.0
TC26095	283	532	336	1142	776	909	-3.0
TC16932	125	188	91	490	284	323	-3.0
TC22052	100	118	149	375	356	323	-3.0

3NSDOCID: <WO_____0112851A2_I_>

Table13. Genes upregulated by aging in C57BL/6 mice heart from Mu6500 GeneChip

ORF	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
X60103	242	223	238	13	-52	65	11.8
AA117446	273	512	453	155	118	66	6.8
M21829	82	83	141	24	45	52	5.4
L07297	69	103	101	-52	-30	-43	5.1
X94998	208	168	223	-8	-35	80	5.1
W36875	149	126	153	15	64	64	4.9
U00677	171	108	187	18	77	5	4.3
M17440	311	354	372	90	84	61	4.0
U08210	45	24	38	-10	4	-17	3.9
AA097087	326	628	684	140	181	143	3.5
X62622	180	134	235	81	112	27	3.5
U25844	702	607	584	186	204	191	3.3
D13664	218	202	130	40	75	75	3.3
U00674	55	48	15	-9	11	15	3.3
Z31663	0	63	55	-42	-100	-88	3.2
X91824	155	121	140	58	60	69	3.2
AA152695	38	42	26	8	8	14	3.2
AA014024	111	219	218	110	59	72	3.1
D16497	1888	1428	3023	664	996	517	3.1
AA036050	52	52	49	18	9	9	3.1
L41154	408	305	476	128	152	157	3.1
AA168633	585	654	733	167	253	246	3.1
L20276	1761	1059	1201	260	600	829	3.0

BNSDOCID: <WO_____0112851A2_I_>

Table 14. Genes downregulated by aging in C57BL/6 mice heart from Mu6500 GeneChip

ORF	oc4	oc5	oc6	yc1	yc2	yc3	Fold Change
X54149	52	16	-69	106	139	84	-6.2
X98475	-7	37	38	202	136	79	-6.1
U25114	185	133	69	326	301	283	-5.4
U58885	-16	33	105	315	212	301	-5.3
X85169	-1	-32	-75	48	43	11	-5.0
AA028728	68	-19	17	90	99	116	-4.9
D14336	100	17	26	141	202	176	-4.8
W29790	72	91	13	259	196	195	-4.8
L11163	181	334	-18	401	820	512	-4.5
AA068712	18	-12	-15	61	69	70	-4.5
D43643	26	-12	-58	69	61	45	-4.3
Y08361	35	1	-35	88	54	84	-4.2
W57425	-6	-31	-61	36	9	13	-4.2
L17076	130	103	97	645	491	431	-4.1
U08215	45	27	-1	160	74	73	-3.8
AA068780	28	-5	-34	86	32	64	-3.8
AA072334	66	43	88	194	160	136	-3.7
AA060808	98	30	57	226	159	155	-3.7
W84060	15	36	6	56	91	63	-3.7
X97796	16	5	-24	72	53	37	-3.6
X60831	49	35	7	52	59	84	-3.6
AA003162	152	28	108	274	204	224	-3.6
W08293	174	130	106	508	356	342	-3.5
AA107999	47	6	-18	77	72	56	-3.5
Z47205	112	93	21	127	181	253	-3.3
AA107137	46	-19	-31	87	165	125	-3.2
U70017	34	0	3	126	63	48	-3.2
W34891	0	19	19	41	40	36	-3.2
M90364	141	94	103	394	273	326	-3.1
W20652	26	43	38	75	63	84	-3.1
W10926	48	-1	-5	99	34	82	-3.1
X53532	13	14	15	92	36	57	-3.0
W77701	167	90	68	369	347	251	-3.0
U53455	22	29	24	127	62	85	-3.0
U09218	17	22	2	57	71	29	-3.0
D78141	29	24	5	54	74	65	-3.0

INSDOCID: <WO_____0112851A2_I_>

Table 15. Genes upregulated by aging in C57BL/6 mice gastrocnemius from Mu19K GeneChip

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC22507	1496	5100	4680	-861	-868	2232	12.3
TC41260	2271	2776	1202	345	337	214	7.1
TC15427	3952	6832	4863	392	2541	1658	6.2
TC17528	309	830	202	-401	-87	58	4.8
TC39719	467	1194	956	-96	-68	639	4.6
TC30023	3484	1557	2722	-471	784	-100	4.2
TC15105	2869	2887	744	424	221	-401	4.2
TC22814	9874	12120	6784	1463	3030	4227	4.2
TC32898	3770	1780	2282	1470	299	598	4.0
TC17624	932	1910	1154	96	704	295	3.9
TC38243	3651	2564	2668	2227	1427	370	3.3
TC32537	2652	2455	3025	723	614	1165	3.3
TC16833	1263	1056	635	427	417	-26	3.1
TC37853	655	965	895	237	151	275	3.1
TC35747	768	1198	1174	477	809	145	3.0
TC36248	3727	6677	4613	2357	2860	1045	2.9
TC16809	2167	1306	1781	648	1219	566	2.8
TC37410	1198	1044	612	564	545	38	2.8
TC29110	1462	775	696	-808	-441	-1038	2.7
TC41340	615	744	603	435	182	403	2.7
TC20762	1280	839	1046	582	553	149	2.7
TC41486	2628	3390	2900	754	2234	1251	2.7
TC30327	3780	2597	2167	628	1606	1354	2.6
TC41030	402	383	450	125	-70	-187	2.6
TC37927	1283	1988	419	-684	-704	-690	2.5
TC35232	206	291	846	-414	-154	-217	2.5
TC40552	676	624	566	180	272	-14	2.5
TC35879	761	606	643	217	248	316	2.5
TC36106	553	81	381	35	-28	-309	2.4
TC14958	431	569	687	37	86	338	2.4
TC15563	1782	2034	1615	779	1031	423	2.4
TC37009	5627	4674	6716	3156	3535	2177	2.4
TC38613	14275	16183	14699	6963	8380	4717	2.4
TC17122	5461	6072	4547	2524	2633	1687	2.4
TC27769	44054	58886	54326	31194	27436	14076	2.4
TC33822	6543	3341	4435	1353	2737	2536	2.4
TC20391	102	324	227	-201	-286	-15	2.4
TC38653	687	826	298	244	59	122	2.4
TC40473	533	539	263	57	118	124	2.3
TC17622	1714	1541	1071	926	397	609	2.3
TC18112	756	793	703	610	211	251	2.3
TC19062	2563	4000	2391	1565	2019	1229	2.3
TC16585	4312	3985	4720	2520	2316	1346	2.3
TC37317	726	1068	673	494	398	258	2.3
TC40165	817	869	775	448	588	182	2.2
TC21714	1174	1390	1120	808	475	702	2.2
TC17422	31965	35070	40903	13173	19477	14605	2.2
TC37018	592	437	367	217	172	79	2.2
TC16885	2486	2538	923	-830	765	-522	2.2
TC34291	13707	19389	10341	8383	5255	6989	2.2
TC37463	1444	1417	1078	922	520	513	2.2
TC24549	8515	9554	5391	4618	4038	3446	2.2
TC35324	321	607	357	140	137	156	2.1
TC31058	1436	1266	1773	514	303	159	2.1
TC15920	2072	2001	1360	477	1197	609	2.1

BNSDOCID: <WO_____0112851A2_I_>

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC29793	1532	1993	2224	458	1173	801	2.1
TC37926	2769	2562	1750	865	1108	1169	2.1
TC40454	1344	2480	2437	590	1123	786	2.1
TC17515	3386	4354	3900	2340	2892	1179	2.1
TC35819	2072	2558	2188	1248	1174	959	2.1
TC39079	1639	1879	1394	538	1352	726	2.1
TC35125	1031	714	880	300	652	40	2.0
TC40951	11	565	108	-204	-192	-530	2.0
TC37262	680	922	706	269	530	3	2.0
TC31287	2040	2088	2058	336	1232	1246	2.0
TC40137	334	303	464	69	135	144	2.0
TC31251	1652	1328	1412	654	696	592	2.0
TC31522	6212	5990	6621	3005	3336	4224	2.0
TC37833	1464	1782	872	587	766	423	2.0
TC23026	462	265	318	105	88	74	2.0
TC33710	5381	4005	5984	1782	3214	2638	2.0
TC14237	978	1638	1423	877	412	747	2.0
TC32046	2438	2103	1415	898	512	1318	2.0
TC15245	2305	2606	4096	1771	1589	503	2.0
TC30375	15067	24645	27999	11194	14149	9870	2.0
TC24289	383	454	679	143	283	-134	2.0
TC30683	1269	622	565	-320	97	122	2.0

INSDOCID: <WO_____0112851A2_I_>

Table 16. Genes downregulated by aging in C57BL/6 mice gastrocnemius from Mu19K GeneChip

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC39172	282	384	1189	1388	1492	1767	-8.6
TC24050	-1117	-243	252	388	1315	2392	-6.8
TC34953	3835	5266	6073	35656	21430	31766	-6.3
TC34306	1324	565	-353	1427	2241	3278	-5.6
TC26537	3726	2008	378	6454	4146	9861	-5.2
TC35355	245	-492	187	765	951	1217	-4.9
TC40742	-394	229	395	1281	1132	1041	-4.7
TC24501	152	253	-108	981	536	1084	-4.6
TC14421	419	1398	344	2366	1833	2615	-4.5
TC21687	-959	88	1433	2686	2066	2732	-4.5
TC25229	369	-201	79	1383	638	1283	-4.2
TC34953	379	2950	2267	5359	3465	5921	-3.9
TC24344	473	528	359	1189	1506	2141	-3.7
TC33957	4504	2776	5281	12197	14665	15262	-3.6
TC40061	4693	1355	4866	7669	10158	7310	-3.5
TC36858	-65	113	276	904	449	854	-3.3
TC15621	3342	3801	2088	5802	5651	7667	-3.1
TC22866	2973	2064	3961	6385	9965	9570	-3.1
TC36347	1077	2585	1662	4287	6166	4493	-3.0
TC26944	13744	8497	7171	26871	31183	24244	-3.0
TC36854	-679	139	-105	2255	4600	2220	-2.9
TC32868	-194	501	-963	1491	1485	569	-2.9
TC33934	-2432	4016	2471	8604	6093	6420	-2.9
TC34857	819	360	-165	2160	2933	3161	-2.9
TC37125	1946	486	1276	2675	2376	2256	-2.7
TC34321	1133	1989	1051	2901	3233	3270	-2.6
TC35099	1565	3225	2314	3774	5816	7280	-2.6
TC22794	420	153	343	1106	1654	1016	-2.6
TC28206	-519	-812	-715	778	784	816	-2.5
TC17374	44879	40619	41419	95128	124767	111416	-2.5
TC19536	38	165	264	626	476	617	-2.5
TC39309	708	927	1767	2405	2161	1651	-2.5
TC14511	2772	859	1861	2932	4587	3089	-2.4
TC25977	-125	907	-393	1714	939	1724	-2.4
TC34555	713	2541	2642	3098	3608	4297	-2.4
TC40318	2484	2040	3012	5440	5650	5710	-2.4
TC22050	721	421	545	944	1092	1638	-2.4
TC23531	264	555	298	677	1076	612	-2.4
TC35434	1150	743	1300	2736	2496	1833	-2.4
TC37551	-265	73	-169	118	422	232	-2.4
TC34651	792	2193	2064	3432	3751	4517	-2.3
TC40365	-286	-312	-315	176	172	252	-2.3
TC26535	4580	11925	9572	12361	20086	21438	-2.2
TC25372	12	141	-161	348	276	386	-2.2
TC28752	816	1567	2442	3958	2783	2378	-2.2
TC21901	1491	754	1326	2284	2539	2382	-2.2
TC41250	628	279	660	782	1093	1096	-2.2
TC20836	102	182	514	781	452	820	-2.2
TC39607	1263	1289	765	1277	1861	1895	-2.2
TC33236	1991	2588	3851	5152	4945	5421	-2.1
TC41556	1138	1047	1367	2263	1972	1988	-2.1
TC41884	475	55	193	650	406	693	-2.1
TC31627	606	494	1343	1839	1123	2105	-2.1
TC35120	1298	1479	752	2993	2032	1705	-2.1
TC37978	664	425	875	1444	1620	1546	-2.1

BNSDOCID: <WO_____0112851A2_I_>

Probe Set	oc1	oc2	oc3	yc1	yc2	yc3	Fold Change
TC32191	329	1419	700	2118	1560	2187	-2.0
TC39472	5773	5966	4650	9742	11750	11019	-2.0
TC36773	2894	3313	4085	5414	7595	6159	-2.0
TC38302	459	289	306	621	809	568	-2.0
TC28179	11576	8026	7030	16063	14643	19203	-2.0

3NSDOCID: <WO_____0112851A2_I_>

CLAIMS

We claim:

1. A method of measuring the biological age of a multicellular organism comprising the steps of:

(a) obtaining a sample of nucleic acid isolated from the organism's organ, tissue or cell, wherein the nucleic acid is RNA or a cDNA copy of RNA and

(b) determining the gene expression pattern of a panel of specific sequences within the nucleic acid pool described in (a) that have been predetermined to either increase or decrease in response to biological aging of the organ, tissue or cell, where the gene expression pattern comprises the relative level of mRNA or cDNA abundance for the panel of specific sequences.

2. The method of claim 1 wherein the expression patterns of at least ten sequences are determined in step (b).

3. The method of claim 2 wherein the expression patterns of at least 20 sequences are determined in step (b).

4. The method of claim 3 wherein the expression levels of at least 30 sequences are determined in step (b).

5. The method of claim 4 wherein the expression levels of at least 40 sequences are determined in step (b).

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6. The method of claim 5 wherein the expression levels of at least 50 sequences are determined in step (b).

7. The method of claim 1 wherein the organism is a mammal.

8. The method of claim 7 wherein the mammal is slected from the group consisting of humans, rats and mice.

9. The method of claim 1 wherein the nucleic acid is isolated from a tissue selected from the group consisting of brain tissue, heart tissue, muscle tissue, skin, liver tissue, blood, skeletal muscle, lymphocytes and mucosa.

10. The method of obtaining biomarkers of aging comprising the steps of:

(a) comparing a gene expression profile of a young multicellular organism subject's organ, tissue or cells; a gene expression profile from a biologically and chronologically aged subject's organ, tissue or cell; and a gene expression profile from a chronologically aged but biologically younger subject's organ, tissue or cell, and

(b) identifying gene expression alterations that are observed when comparing the young subjects and the chronologically and biologically aged subjects and are not observed or reduced in magnitude when comparing the young subjects and chronologically aged but biologically younger subjects.

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11. The method of claim 10 wherein one uses high density oligonucleotide arrays comprising at least 5-10% of the subject's genes to compare the subjects gene expression profile.

12. The method of claim 10 wherein the gene expression profile indicates a two-fold or greater increase or decrease in the expression of certain genes in chronologically aged subjects.

13. The method of claim 10 wherein the gene expression profile indicated a 3-fold or greater increase or decrease in the expression of certain genes in chronologically aged subjects.

14. The method of claim 10 wherein the gene expression profile indicates a 4-fold or greater increase or decrease in the expression of certain genes in chronologically aged subjects.

15. A method of measuring biological age of muscle tissue comprising the step of quantifying the mRNA abundance of a panel of biomarkers selected from the group consisting of markers W08057, AA114576, 11071777, 11106112, D29016, and M16465.

16. A method of measuring biological age of muscle tissue comprising the step of quantifying the mRNA abundance of a panel of biomarkers selected from the group consisting of markers described in Tables 1, 2, 15, and 16.

17. A method of measuring biological age of brain tissue comprising the step of quantifying the mRNA abundance of a panel of

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biomarkers selected from the group consisting of markers M17440, K01347, AA116604 and X16995.

18. The method of claim 10 wherein the subject is a mammal.

19. The method of claim 18 wherein the mammal is selected from the group consisting of humans, mice and rats.

20. A method of measuring biological age of brain tissue comprising the step of quantifying the mRNA abundance of a panel of biomarkers selected from the group consisting of markers described in Tables 5, 6, 9, and10.

21. A method of measuring biological age of heart tissue comprising the step of quantifying the mRNA abundance of a panel of biomarkers selected from the group consisting of markers described in Tables 11, 12, 13 and 14.

22. A method for screening a compound for the ability to inhibit or retard the aging process in multicellular organisms tissue, organ or cell comprising the steps of:

(a) dividing test organisms into first and second mammalian samples;

(b) exposing the organisms of the first sample to a test compound;

(c) analyzing tissues, organs or cells of the first and second samples for the level of expression of a panel of sequences that have been

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predetermined to either increase or decrease in response to biological aging of the tissue;

(d) comparing the analysis of the first and second samples and identifying test compounds that modify the expression of the sequences of step (c) in the first sample such that the expression pattern is indicative of tissue, organ or cell that has an inhibited or retarded biological age.

23. A method as in claim 22, wherein the organism is a mammal.

24. The method of claim 23, wherein the mammal is selected from the group consisting of humans, rats and mice.

25. A method as in claim 23, wherein the tissue is selected from the group consisting of brain tissue, heart tissue, muscle tissue, blood, skeletal muscle, mucosa, skin, lymphocytes and liver tissue.

26. A method of detecting whether a test compound mimics the gene profile induced by caloric restriction, comprising the steps of:

(a) exposing a multicellular organism to the test compound, and

(b) measuring the expression level of a panel of sequences predetermined to either increase or decrease in response to biological aging in a tissue, organ or cell of the organism and comparing the measurement to a measurement obtained in the same tissue, organ or cell in calorically restricted subjects.

27. The method of claim 26 wherein the multicellular organism is a mammal.

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28. The method of claim 27 wherein the mammal is selected from the group consisting of humans, rodents and mice.

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(54) Title: IDENTIFICATION OF GENETIC MARKERS OF BIOLOGICAL AGE AND METABOLISM

(57) Abstract: A method of measuring the biological age of a multicellular organism is disclosed. In one embodiment this method comprises the steps of obtaining a sample of nucleic acid isolated from the organism's organ, tissue or cell and determining the expression pattern of a panel of sequences within the nucleic acid that have been predetermined by either increase or decrease in response to biological aging of the organ, tissue or cell. A method of obtaining biomarkers of aging is also disclosed. This method comprises the step of comparing a gene expression profile of a young multicellular organism subject's organ, tissue or cells; a gene expression profile from a chronologically aged subject's organ, tissue or cell; and a gene expression profile from a chronologically aged but biologically younger subject's organ, tissue or cell and identifying gene expression alterations that are observed when comparing the young subjects and the chronologically aged subjects and are not observed or reduced in magnitude when comparing the young subject and the chronologically aged but biologically younger subjects.

BNSDOCID: <WO_____0112851A3_I_>

INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/US 00/21603

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MALCOLM H. GOYNS ET AL.: "Differential display analysis of gene expression indicates that age-related changes are restricted to a small cohort of genes" MECHANISMS OF AGEING AND DEVELOPMENT, vol. 101, no. 1,2, 16 March 1998 (1998-03-16), pages 73-90, XP000990037	1-14, 17-20, 22-25
Y	abstract page 78, paragraph 3 -page 89, last paragraph	26-28
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2001	02/04/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

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INSDOCID: <WO_____0112851A3_I_>

INTERNATIONAL SEARCH REPORT

Inte. .onal Application No
PCT/US 00/21603

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 13610 A (GERON CORPORATION) 31 October 1994 (1994-10-31) page 8, paragraph 2 -page 9, paragraph 1 page 10, last paragraph -page 11, paragraph 1 page 12, paragraph 2 -page 15, paragraph 2 page 18, paragraph 2 -page 21, paragraph 2; tables 2,3 page 54, paragraph 3 ---	1-14,18, 19,22-25
X	BANDANA CHATTERJEE ET AL.: "Differential regulation of the messenger RNA for three major senescence marker proteins in male rat liver" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 256, no. 12, 25 June 1981 (1981-06-25), pages 5939-5941, XP000990218 abstract page 5939, right-hand column, paragraph 5 -page 5941, left-hand column, paragraph 1 ---	1-14,18, 19,22-25
X	CHERYL WISTROM ET AL.: "Cloning and expression of SAG: A novel marker of cellular senescence" EXPERIMENTAL CELL RESEARCH, vol. 199, no. 2, April 1992 (1992-04), pages 355-362, XP000099098 abstract page 355, right-hand column, paragraph 4 -page 356, left-hand column, paragraph 2 page 357, left-hand column, paragraph 3 -page 358, left-hand column, paragraph 1 ---	1-14,18, 19,22-25
X	MARIA TRESINI ET AL.: "Effects of donor age on the expression of a marker of replicative senescence (EPC-1) in human dermal fibroblasts" JOURNAL OF CELLULAR PHYSIOLOGY, vol. 179, April 1999 (1999-04), pages 11-17, XP000990062 abstract page 14, left-hand column, paragraph 2 -page 16, left-hand column, paragraph 2 ---	1-14,18, 19
Y	ROY L. WALFORD ET AL.: "Dietary restriction and aging: historical phases, mechanisms and current directions" JOURNAL OF NUTRITION, vol. 117, no. 10, October 1987 (1987-10), pages 1650-1654, XP000990310 the whole document --- -/--	26-28

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BNSDOCID: <WO_____0112851A3_I_>

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 00/21603

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHEOL-KOO LEE ET AL.: "Gene expression profile of aging and its retardation by caloric restriction" SCIENCE, vol. 285, no. 5432, 27 August 1999 (1999-08-27), pages 1390-1393, XP002162927 DC cited in the application the whole document	1-28
P,X	CHEOL-KOO LEE ET AL.: "Gene-expression profile of the ageing brain in mice" NATURE GENETICS, vol. 25, no. 3, July 2000 (2000-07), pages 294-297, XP000990091 cited in the application the whole document	1-14, 17-20, 22-28

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NSDOCID: <WO_____0112851A3_I_>

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: partially 16 and 21

Present claims 16 and 21, when referring to tables 11, 12, 15 and 16 relate to compounds defined by reference to "TC" designations. The use of these designations in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the internal designations the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to the compounds in tables 1, 2, 11 and 12 defined by accession numbers.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/US 00/21603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9613610 A	09-05-1996	US 5744300 A	28-04-1998
		AU 698841 B	12-11-1998
		AU 3692495 A	23-05-1996
		EP 0789780 A	20-08-1997
		JP 10508200 T	18-08-1998

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